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Transcriptomic analysis of the response of the lactic acid bacteria *Lactococcus piscium* for the identification of possible adaptive genes during thermal shock in cold and warm temperatures.

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<p>Tiivistelmä – Referat – Abstract</p> <p>Psychrotrophic lactic acid bacteria (LAB) play a versatile role in research, food, farming and medicinal applications, but also play a role as a source of food spoilage. The effects of temperature changes has yet to be studied in depth.</p> <p>In this study, to analyze in a transcriptome level, cold and heat shock stress to spoilage lactic acid bacteria, a time-dependent RNA-seq for <i>Lactococcus piscium</i> with a temperature of 0 °C and 28 °C was conducted.</p> <p>The data of protein regulation during the experiment shows that <i>Lactococcus piscium</i> has the essential machinery to survive against different types of environmental stress. I observed known heat shock related genes and stress related genes to be present in the regulated response of both temperature extremes. Cold shock upregulation is observed after 35 minutes, which could indicate that the metabolic response at cold temperatures is related to growth rate. With a clear downregulation of pathways of energy consumption and an adaptation in terms of RNA being more prominent than at 28 °C. Certain surface, cell wall and transport proteins are noticeable more upregulated at 0 °C in addition to a downregulation of energy related proteins, which in correlation with its growth curve, we can assume it is part of a mechanism of protection against cold environments, in comparison with the regulation of proteins at 28 °C. This gives an insight of a well controlled preservation mechanism that <i>Lactococcus piscium</i> exhibits that can be linked to its environment.</p> <p>The findings of this research offer new understandings into the survival skills of <i>Lactococcus piscium</i> to a cold and heat shock. Future studies should focus on analyzing the regulation and function of possible new genes for the response to heat stress, as well as the specific function of proteins related to temperature stress in the events where change of temperature plays a constant role in the environment.</p>			
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Introduction:

The use of microorganisms to expand shelf life of food products dates to ancient times. Lactic acid bacteria (LAB) play a versatile role in research, food, farming and medicinal applications[1, 2]. LAB is a group of microorganisms with the ability to transform food through fermentation, in addition to being transformers of the synthesis of metabolites used for the benefit of humans, such as lactic acid or short chain peptides that have antimicrobial properties [3].

The interactions between lactic bacteria and food have been studied and categorized for more than a century. Fermentation processes are associated with different types of specific microorganisms. In 1986, David Hendricks Bergey, published in his book: Manual of Systematic Bacteriology [4], different genus for the classification of gram-positive lactic acid bacteria, cocci or bacilli, not sporulated, with facultative anaerobic respiration; which resulted in a list of seven genus. This classification has presented a large number of changes over the years, which has caused some confusion in the taxonomy but at its core, the genera of the LAB is comprise of: Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, and Streptococcus, as well as Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Tetragenococcus, Vagococcus, and Weissella.

The Lactococcus genus has been extensively studied among LAB, as certain species are of significant economic interest for the food industry [5]. Lactococcus is a genus of Gram-positive, pleomorphic bacteria that can grow individually, in pairs, or on chains. They are homofermentative chemoorganotrophs and have the capacity to generate lactic acid from glucose. Some strains are usually utilized in manufacturing processes, like dairy starter cultures [6] and probiotics in food products [7]. The food industry employs some species of the genus [8]. They are generally considered non-pathogenic or opportunistic pathogenic [9]. Historically, LAB have been associated as food-altering bacteria in dairy and meat products. However, LAB is now known that it can be used in the food industry to prolong its consumption time, as well as preserve and or transform the characteristics that improve the microbiological and food quality

of the product due to its antagonistic action towards organisms that can cause food spoilage [10-13].

The sensory characteristics, life span, and safety of most fermented foods are controlled by the metabolic processes [14] and the changes that lactic acid bacteria can cause to the environment were its present. In frozen food, meat products with a modified atmosphere package, LAB is part of the large portion of the microbiota present [15-18]. The sensitivity and ability to tolerate various environmental changes of LAB makes them of considerable significance for the food industry [19].

To determine the quality of food, we inspect different characteristics, such as color, odor, taste and texture. If one of these characteristics is not met, the product is most likely unfit for human consumption and has to be discarded and destroyed. This results in large economic losses and is a significant concern for the food industry, that is why cold adaptation is an important advantage of bacterial competition in food products that usually are frozen to extend shelf life, in particular against spoilage and pathogenic psychrotrophs [20, 21].

The introduction of microorganisms, like *Lactococcus piscium*, has been part of strategies to avoid or delay food degradation that had been involved in the manufacturing process [22-26], in conjunction to many other techniques [27]. *L. piscium* was first described in 1990 [28]. This microorganism is psychotropic [12], that means that it exhibits an unusual growth profile linked to temperature. The development, as opposed to most of the LAB, occurs between 0 °C and 29 °C, with an optimal temperature of 25 °C, and is hindered at temperatures above 29 °C, showing a particular behavior of cold adaptation [29], but has also been associated with spoilage of poultry, seafood and, to a lesser degree, vegetable goods [30, 31], that why it requires identifying their adaptation advantages and challenges, as they attain elevated thresholds that outcompete the cold-adapted microbes used, to understand key aspects of their adaptation to a genetic level.

In this study, *Lactococcus piscium* was analyzed to explore its response at different temperatures, creating controlled stress at both high and low temperatures. This psychrotrophic microorganism that is commonly found in refrigerated meat products

and with a connection to spoilage, that is why the evaluation of the adaptive response is important in the preservation of susceptible products. The result obtained is a characterization of the reaction of this bacterium to drastic changes in temperature, managing to observe at a genetic level what its response is under thermal stress.

Materials and Methods:

Experimental Set Up and Sampling

Bacteria Material

One LAB strain was used in this experiment: *Lactococcus piscium* MKFS47. The bacteria cultures were grown in four plates; Man-Rogosa-Sharpe (MRS) (-) acetate, M17 (lactose) + polymyxin-B 60 µg/ml (selective for *L. piscium*), MRS (glucose) + fusidic acid 30 µg/ml and MRS (xylose) + kanamycin 90 µg/ml.

Bacteria Preparation

Lactococcus piscium

Frozen stock (1.8 ml) was added to 9 ml of MRS(-) acetate medium, leaving it overnight at 25 °C (in temperature cabin) for growth. Making a dilution 1:10, I measure the OD₆₀₀, and plated 1 µl onto 4 MRS(-) acetate culture plates: MRS(-) acetate, M17 (lactose) + polymyxin-B 60 µg/ml (selective for *L. piscium*), MRS (glucose) + fusidic acid 30 µg/ml, and MRS (xylose) + kanamycin 90 µg/ml, with anaerobic conditions for growth over 3 nights at 25 °C. As expected, the microorganism growth was present only in the MRS(-) acetate and in the selective medium plate. I took one colony of each plate and inoculated a tube with 9 ml of MRS(-) acetate medium, leaving it for overnight growth at 25 °C. The next day, making a dilution 1:10, we measured the OD₆₀₀ and again, we inoculated a 9 ml MRS(-) acetate tube, with 100 µl of the previous tubes. We measure OD₆₀₀ (dilution 1:10) of both tubes, looking for an absorbance close to 1, to inoculate three 100 ml growth media bottles, previously set at a 25 °C, with 10 µl, 30 µl and 90 µl of the measured selected sample, leaving it overnight at the

temperature cabin. Early on the next day, we measure the OD_{600} , looking for an absorbance between 0.2–0.4 (dilution 1:10) in one of the cultures, which would be selected to start the heat/cold experiment. I inoculate four 100 ml growth media bottles with MRS (-) acetate medium, so that the starting OD_{600} value was between 0.2–0.4. After that the growth was followed by measuring the OD_{600} every hour.

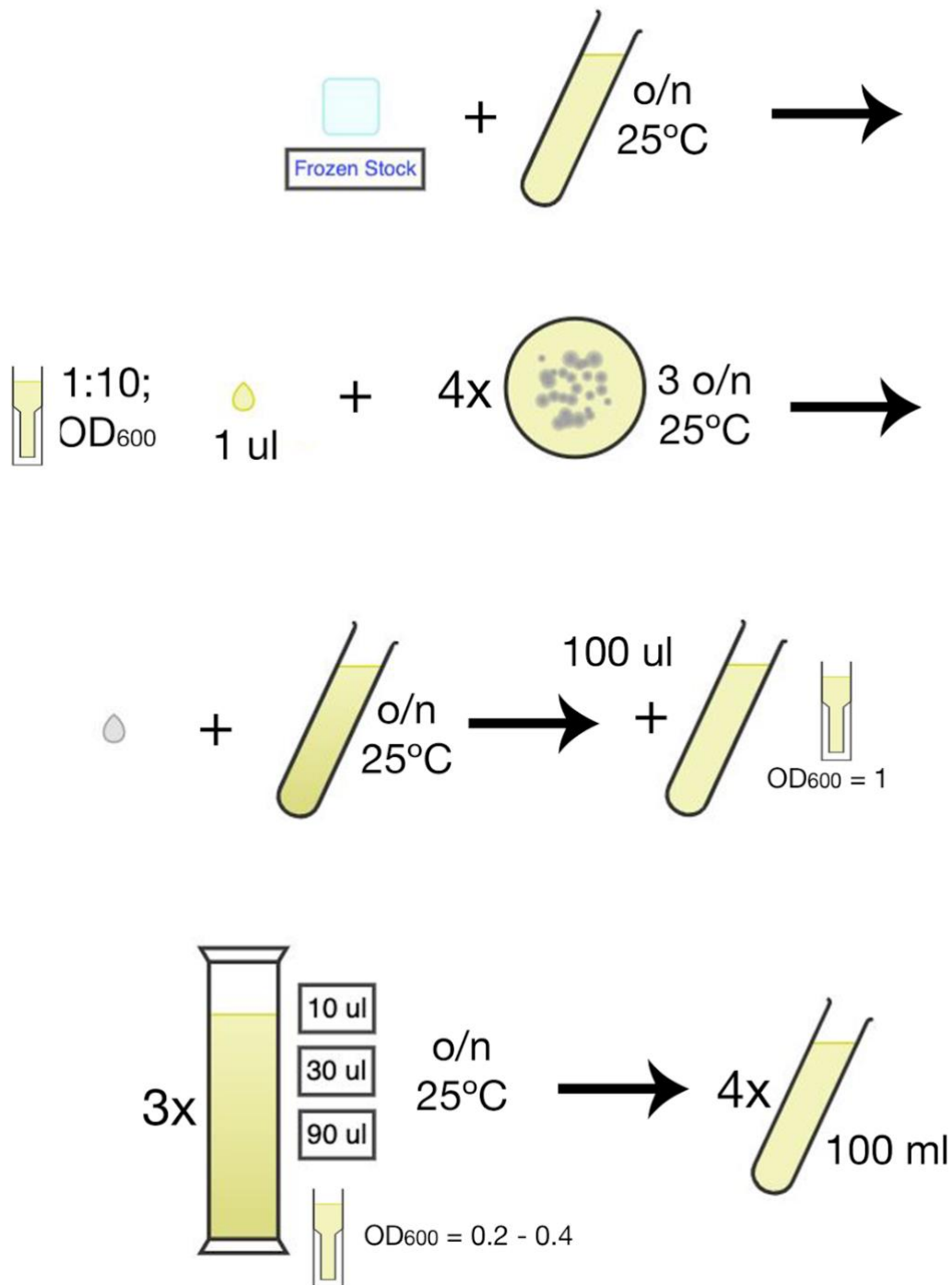


Figure 1. Schematic of the preparation for *Lactococcus piscium* MKFS47 from frozen stock for temperature shock experiment. Bacterial stock was growing on MRS (-) medium overnight at 25 °C. After the growth period, OD₆₀₀ was measured to observe if growth occurred. One microliter was plated in 4 different MRS (-) plates and grown for 3 nights at 25 °C. One resulting colony is growth in liquid MRS (-) medium overnight at 25 °C. After growth period, 100ul are utilized to inoculate a new liquid MRS (-), if OD₆₀₀ is close to 1. Three 100ml growth bottles are then inoculated with 10, 30 and 90 ul and left to grow overnight at 25 °C. The next day OD₆₀₀ is measured and the sample that has an absorbance between 0.2 and 0.4 is then used to inoculate four 100ml tubes, for the temperature shock experiment.

OD₆₀₀

Indicates the absorbance, or optical density, of a sample measured at a wavelength of 600 nm. It is a common method for estimating the concentration of bacterial or other cells in a liquid to indicate the stage of cultured cell population. For this experiment, all OD₆₀₀ calculations were made with Pharmacia Biotech Ultrospec 3000 UV/Visible Spectrophotometer.

Temperature Shock Experiment

For this experiment, I started by the inoculation of four flasks, measuring the OD₆₀₀ hourly until it reaches OD₆₀₀ ≈ 0.5, then 5 aliquots of 10ml were transferred to glass tubes at different temperatures: 0°C, 4 °C, 14 °C, 25 °C and 28 °C, to follow the growth pattern. Samples for RNA extraction were taken after 5, 35 and 185 minutes of incubation. Measures of OD₆₀₀ of the starting 100 ml cultures were continued, to follow the growth. In addition. OD₆₀₀ values that were measured just before starting the temperature experiment, were used to calculate the amount of culture sampled for RNA extraction at 5- and 35-minutes sampling points. OD₆₀₀ was measured from the temperature experiment glass tubes before 185 minutes sampling point, so that we could adjust the sample volume accordingly. Sample volumes were determined using the formula: $V = (1/1.5 \cdot OD_{600})$. The same sample volume was used at 0 min, 5 min and 35 min time points, Samples were mixed with a 1:10 of a volume of ethanol-phenol

mixture (10:1) to stop RNase activity. Cells were precipitated by centrifugation at 5000 x g for 3 min, frozen in liquid nitrogen, and stored in -80 °C.

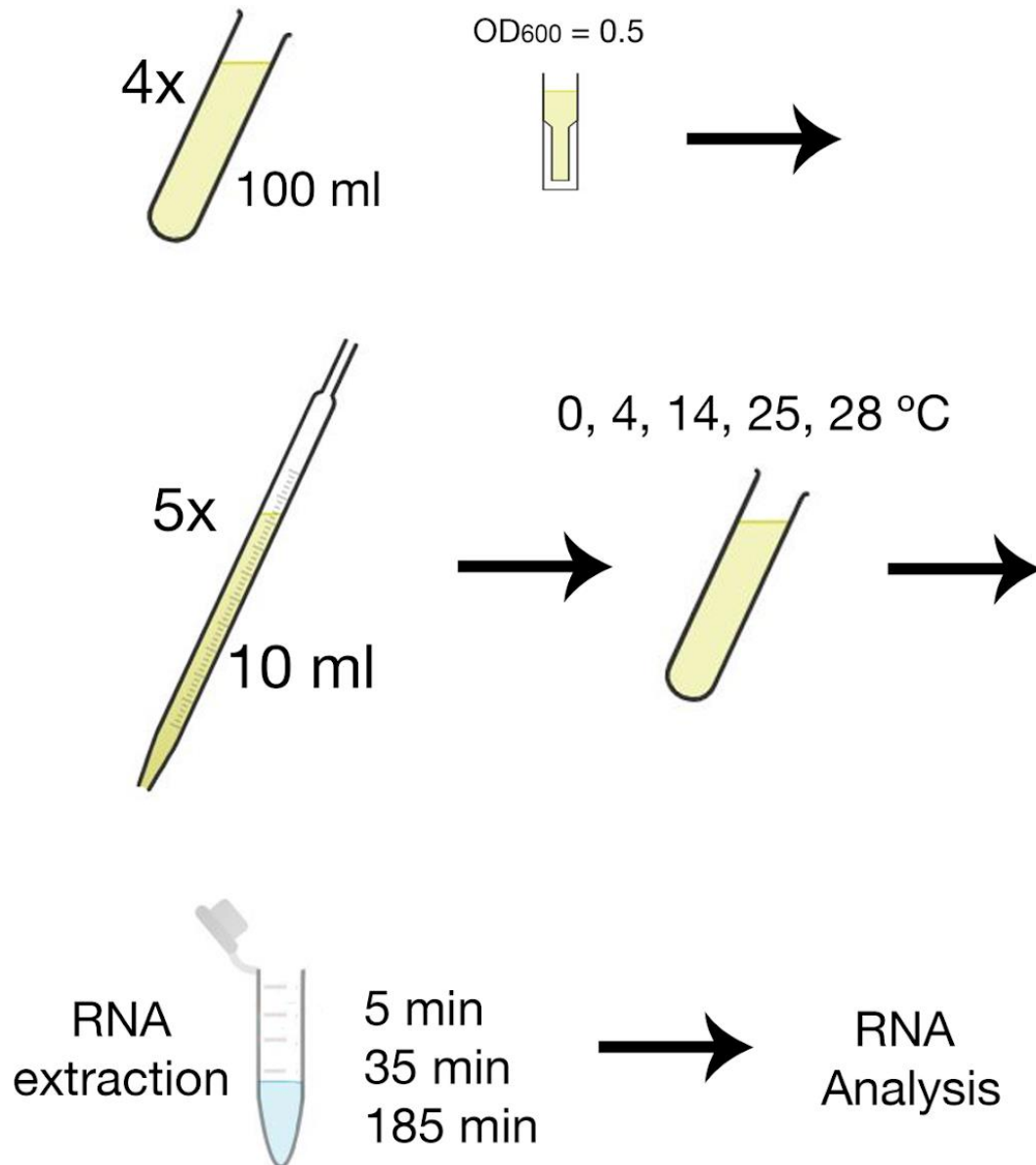


Figure 2. Schematic of the temperature shock experiment of previously prepared samples of *Lactococcus piscium*, for RNA analysis. Samples OD₆₀₀ is measured, looking for an absorbance of 0.5 to then aliquot 10ml to 5 different tubes at temperatures of 0 °C, 4 °C, 14 °C, 25 °C, and 28 °C, for comparison of growth curve, OD₆₀₀ was measured every hour. For RNA extraction, samples were taken at 0 min, 5 min and 35 min time points. RNA samples were then mixed with a 1:10 of a volume of ethanol-phenol mixture (10:1) to stop RNase activity. Cells were precipitated by centrifugation at 5000 x g for 3 min, frozen in liquid nitrogen, and stored in -80 °C.

Transcriptome Sequencing

RNA Extraction

RNA isolation was performed using an RNA Isolation Kit (NucleoSpin RNA; Macherey-Nagel, Düren, Germany), with some modifications in the protocol presented in the manual (RNA preparation from up to 10⁹ bacterial cells) for cell lysis. The RNA isolation was performed in the samples previously taken in the temperature experiments.

Resuspension of frozen pellets was performed with 700 µl of Buffer RA1, moving the resuspension mix to a Lysing Matrix E or B Tube (MP Biomedicals, Irvine, CA, USA). I added 7 µl of β-mercaptoethanol, under fume hood. The resulting mix was vortexed for 5 to 10 seconds and kept on ice until the next step. Cells were disrupted using a FastPrep homogenizer (MP Biomedicals), with settings of 5.5 m/sec, for 40 seconds. During transport cells were kept on ice. Next, cells were centrifuged 30 seconds at maximum speed, at room temperature. The cell lysate was filtered through NucleoSpin Filters in a collection tube (2ml) by centrifuging for 1 minute at 11,000x g. We measured the volume of the filtered lysate and transferred it to a new 1.5-ml tube; I added the same volume of EtOH (70%) to the lysate and vortexed it vigorously. Using a NucleoSpin RNA Column in a collection tube, we filtered the lysate to bind the RNA in the column. I centrifuged the complete volume at 11,000x g and then we changed the collection tube, discarding the filtered liquid. Following the manufacturer's instructions, I added 350 µl MDB to column and centrifuged 1 minute (11 000 x g). To digest the bound DNA, I then incubated the column, adding 95 µl of DNase mix (10µl reconstituted rDNase + 90 µl Reaction Buffer for rDNase) for 15 minutes at room temperature. After the incubation, I added 200 µl Buffer RAW2 and centrifuged for 30s

(11,000x g), changing the collection tube afterwards. I then added 600 µl Buffer RA3 and centrifuged for 30 s (11,000x g), discarded flow-through and kept the same collection tube. Lastly, I added 250 µl Buffer RA3 and centrifuged for 2 minutes (11,000x g), and placed the column in a new collection tube (1.5 ml). The RNA was eluted by adding 60 µl of RNase-free water, and centrifuging 1 minute at 11,000x g. We can discard the column after this step. The RNA solutions obtained were stored at -80 °C.

RNA analysis

To evaluate the integrity of our RNA, I used The Agilent 2100 Bioanalyzer System using the RNA Nano Kit (Agilent, Santa Clara, CA, USA). This system uses electrophoretic separation in chips, where the samples are separated and detected via laser-induced fluorescence detection. As a result, the system generates an electropherogram of the sample, showing the concentration and the RNA integrity number (RIN), which allows us to observe the quality of the RNA in our sample. RNA analysis was performed with one µl of each RNA sample.

rRNA removal for NGS Library

rRNA accounts for a high percentage of all NGS library inserts when total RNA is used as raw material for library making. To obtain rRNA depleted RNA, different methods were compared before proceeding to the next step of library making.

RNaseH + pH 6.8 buffer

RNA is used as raw material for library making. To obtain rRNA depleted RNA I first hybridize oligos to RNA, this was done by mixing a volume of the sample, in µl, calculating a concentration of 1000 ng, 1 µl of 10x buffer (pH6.8), and 1 µl of 3 µM oligo pools, adjusting the volume to 7 µl with RNA-free water. The mix was heated for two minutes at 95°C, with slow cool (0.1 °C/s) to 22 °C; holding the sample at 22°C for 5 minutes, placing in ice immediately after. I then digest our rRNA, by adding to the hybridized RNA sample (7 µl) 2.0 µl of RNaseH (10 u/µl or 5 u/ µl) (Thermo Fisher

Scientific, Waltham, MA, USA), 1.5 μ l of 0.1 M DTT, 0.6 μ l of 25 mM $MgCl_2$ and 0.5 μ l of Ribolock RNase Inhibitor (Thermo Fisher Scientific), adjusting volume to 15 μ l with RNA-free water. The mix was incubated 60 minutes at 37 °C. To inactive the digestion, 20 minutes at 65 °C. The next step was the removal of DNA oligos, which was made with the RapidOut DNA Removal Kit (Thermo Fisher Scientific), following the protocol provided by the manufacturer's instructions, changing the final volume of the mix to 20 μ l, to accommodate the 15 μ l of RNA sample. I added 2 μ l of 10x DNase buffer, 1 μ l of DNase I, 0.5 μ l of RNase Inhibitor, and adjusted to the volume aforementioned with DNA-free water. We follow with an incubation at 37 °C for 30 minutes. Then 2 μ l DNase removal reagent (DRR) was added, with an incubation of two minutes at room temperature, followed by a centrifugation at 1000 x g for one minute to pellet DRR and beads, and recover supernatant to a new tube.

RNaseH + pH 8 buffer

Repeating the process of RNaseH + pH 6.8 buffer, changing the aforementioned buffer for the new one. Following next step as previous method. For the step of rRNA digestion, I added to the hybridized RNA sample (7 μ l) 2.0 μ l of RNaseH (10 u/ μ l or 5 u/ μ l) (Thermo Fisher Scientific), 0.15 μ l of 0.1 M DTT, 1.2 μ l of 25 mM $MgCl_2$ and 0.5 μ l of Ribolock RNase Inhibitor (Thermo Fisher Scientific), adjusting volume to 15 μ l with RNA-free water. The next steps are followed as normal.

Thermo Fisher RNaseH

Just as the previous methods, RNA is used as raw material for library making. To obtain rRNA depleted RNA I mixed a volume of the sample, in μ l, calculating a concentration of 1000 ng, 1 μ l of 10x buffer (Thermo Fisher Scientific), and 1 μ l of 3 μ M of oligo pools, adjusting the volume to 12 μ l with RNA-free water. Following next step as previous methods.

For the step of rRNA digestion, I added to the hybridized RNA sample (12 μ l) 2.0 μ l of RNaseH (5 u/ μ l) (Thermo Fisher Scientific), 1.5 μ l of 10x Buffer (Thermo Fisher

Scientific) and 0.5 µl of Ribolock RNase Inhibitor (Thermo Fisher Scientific), adjusting volume to 15 µl with RNA-free water. The next steps are followed as normal.

Thermo Fisher RNaseH + pH 8 buffer

We follow the procedure of RNaseH + pH 6.8 buffer. To obtain rRNA depleted RNA we mix a volume of the sample, in µl, calculating a concentration of 1000 ng, 1.5 µl of 10x buffer (pH8), and 1 µl of 3 µM of oligo pools, adjusting the volume to 7 µl with RNA-free water. Following next step as previous methods.

For the step of rRNA digestion, I added to the hybridized RNA sample (7 µl) 0.2 µl of RNaseH (10 u/µl or 5 u/ µl) (Thermo Fisher Scientific), 0.15 µl of 0.1 M DTT, 1.2 µl of 25 mM MgCl₂ and 0.5 µl of Ribolock RNase Inhibitor (Thermo Fisher Scientific), adjusting volume to 15 µl with RNA-free water. The next steps are followed as normal.

Ribo-Zero rRNA

Ribosomal RNA removal was performed with Ribo-Zero rRNA removal reagent for bacteria (Illumina, San Diego, CA, USA) according to manufacturer's instructions. I first hybridize oligos to RNA, this was done by mixing 1/3 volume of the sample, in µl, calculating of 1000 ng of total RNA, rRNA-depleted RNA was purified with RNA Clean&Concentrator -5 kit (Zymo Research, Irvine, CA, USA) according to manufacturer's instructions and eluted in 11 µl of RNA-free water.

Library Preparation

Two methods were compared before proceeding to the next step of sequencing data processing.

QIAseq Library

For the NGS libraries, QIAseq Stranded Total RNA Lib Kit (Qiagen, Hilden, Germany) was used, following the manufacturer's instructions for whole transcriptome next-

generation sequencing of RNA, with the exception of the last step of CleanStart library amplification, which was not done in this experiment.

For the next step, I mixed 10 µl of the template for each sample previously prepared, and adding the libraries were amplified using half (10 µl) of the purified ligation product in 1 x HF buffer with 0.2 mM dNTPs, 0.6 µM selected [22] dual-index primer, and one unit of Phusion Hot Start II High-Fidelity DNA polymerase (Thermo Fisher Scientific). In strand-specific ligation, 0.3 µM truncated Truseq adapter was used instead of the adapter provided in the kit.

The mix then was amplified with PCR protocol, 18 cycles of 98 °C, 30s; 98 °C, 10s; 65 °C, 30s; 72 °C, 10s; followed by 5 minutes at 72°C, leaving at 4 °C when finished. After amplification, an aliquot of 2.5 µl was taken for analysis before pooling all samples. Concentrations of amplified libraries were measured with Qubit fluorometer and dsDNA HS assay kit (Invitrogen, Waltham, MA, USA), and size distributions visualized with Fragment Analyzer and High Sensitivity NGS Fragment Analysis kit (Advanced Analytical, Parkersburg, WV, USA). After pooling, libraries were concentrated using Amicon Ultra 100K columns (Millipore, Burlington, MA, USA), and purified two times with 0.9 x AMPure XP beads (Beckman Coulter, Brea, CA, USA). For the DNA size selection of the obtained libraries, PEG size selection (8% - 8.5%) was used. Illumina NextSeq 500 was used to perform the sequencing of the RNA-seq libraries single end 75bp.

Illumina Library

For the NGS libraries, TruSeq RNA Library Prep Kit V2 (Illumina) was used, following the manufacturer's instructions for whole transcriptome next-generation sequencing of RNA, with the exception of the last step of CleanUp PCR library amplification, which was not done in this experiment.

For the next step, I mixed 10 µl of the template for each sample previously prepared, and adding 10 µl of 5x Buffer, 1 µl of 10 uM PCR_TrusecA, 1 µl 10 uM NTPs, 0.5 µl

of Phusion Hot Start II High-Fidelity DNA polymerase, 1 ul of a selected index primer and raised to 50 ul of final volume with water.

The mix was then amplified with PCR protocol, following the steps aforementioned, and used in the QIAgen protocol. The resulting RNA-seq libraries single end 75 bp, were sequenced with Illumina NextSeq 500.

For the purposes of this research, data processing was done to samples depleted of rRNA with the Ribo-Zero rRNA method and their library prepared with the QIAseq Kit.

Sequencing data processing

Sequencing of the libraries was performed by the personnel of the DNA sequencing and genomics laboratory, of the Institute of Biotechnology at the University of Helsinki.

Results

***Lactococcus piscium* MKFS47**

Bacterial Growth

Exponential (log) phase curve for bacterial growth were observed after a time of incubation of 2 hours for *Lactococcus piscium* in a sample used as a control. Measuring of exponential growth was done for 10 hours. Aliquots were taken and transferred to different tubes with controlled temperatures (0° C and 28° C) when absorbance (OD600) reached an optical density around 0,5. Absorbance was measured at time points 5, 35 and 185 minutes. As expected, the organism presented a slower exponential growth at cold temperature (0° C) compared to the samples at warmer temperatures (28° C), which were compared to the sample at 25° C, used as a control aliquot.

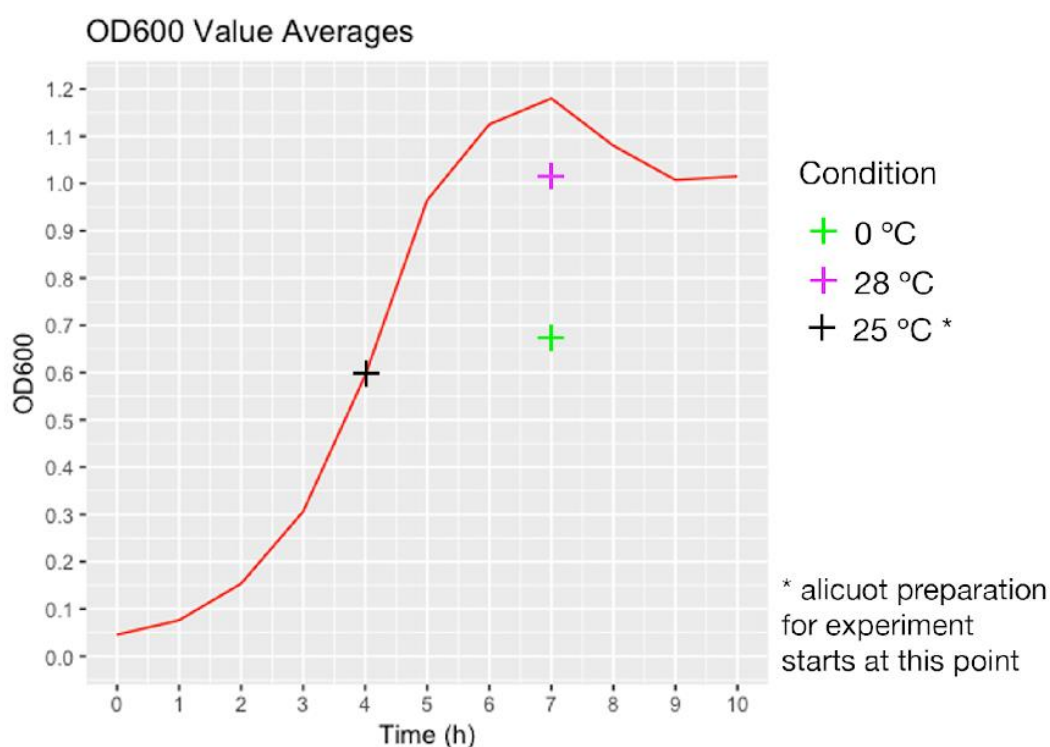


Figure 3. Growth curve of *Lactococcus piscium* based on optical density (OD₆₀₀) values. The red colored curve represents the average growth at 25 °C in liquid medium. Sampling time for aliquots for temperature experiment is marked in the figure with a black cross. Colored points represent averages of samples at different temperatures at 185 min; green: 0 °C, and pink: 28 °C, compared to the control (25 °C) using the student's t-test p-value < 0.05. It is observed that a difference in exponential growth is linked to temperature.

Selecting Samples for Data Processing

As observed with sequencing results (Table 1) Thermo RNaseH worked better than other protocols for rRNA removal, however, there is still room for some improvement that can be done in the future. For this experiment, it was decided that a better choice was to use the commercial rRNA removal method (Ribo-Zero) for the *L. piscium* temperature stress experiment samples.

Table 1. Sequencing results for comparison of rRNA removal methods.

RUN	Enzyme	Buffer	Library	QUALITY FILTERING AND ADAPTER TRIMMING			SORTME RNA (RRNA FILTERING)	FINAL CLEAN READS NUMBER
				Input Reads	Surviving	Dropped		
R1	MF RnaseH	Own, pH8	Illumina	1278579	1244827 (97.36%)	33752 (2.64%)	1156793 (92.93%)	88034 6.89%
R2	MF RnaseH	Own, pH8	Illumina	1278579	1187254 (92.86%)	91325 (7.14%)	1102811 (92.89%)	84443 6.60%
R1	Thermo RnaseH	Thermo	Illumina	1195924	1152487 (96.37%)	43437 (3.63%)	1013544 (87.94%)	138943 11.62%
R2	Thermo RnaseH	Thermo	Illumina	1195924	1092930 (91.39%)	102994 (8.61%)	960378 (87.87%)	132552 11.08%
R1	MF RnaseH	Own, pH8	Qiagen	708684	661896 (93.40%)	46788 (6.60%)	615760 (93.03%)	46136 6.51%
R2	MF RnaseH	Own, pH8	Qiagen	708684	622411 (87.83%)	86273 (12.17%)	579412 (93.09%)	42999 6.07%
R1	MF RnaseH	Own, pH6.8	Qiagen	1049916	938948 (89.43%)	110968 (10.57%)	899454 (95.79%)	39494 3.76%
R2	MF RnaseH	Own, pH6.8	Qiagen	1049916	911681 (86.83%)	138235 (13.17%)	873546 (95.82%)	38135 3.63%
R1	MF RnaseH	Own, pH6.8	Qiagen	649322	517482 (79.70%)	131840 (20.30%)	478995 (92.56%)	38487 5.93%
R2	MF RnaseH	Own, pH6.8	Qiagen	649322	493077 (75.94%)	156245 (24.06%)	456215 (92.52%)	36862 5.68%
R1	Thermo RnaseH	Own, pH8	Qiagen	566731	487957 (86.10%)	78774 (13.90%)	404457 (82.89%)	83500 14.73%
R2	Thermo RnaseH	Own, pH8	Qiagen	566731	469770 (82.89%)	96961 (17.11%)	389216 (82.85%)	80554 14.21%
R1	Thermo RnaseH	Own, pH8	Qiagen	155411	119824 (77.10%)	35587 (22.90%)	95762 (79.92%)	24062 15.48%
R2	Thermo RnaseH	Own, pH8	Qiagen	155411	115693 (74.44%)	39718 (25.56%)	92508 (79.96%)	23185 14.92%

Genetic Expression After Temperature Shock

Temperature shock was done with 2 temperatures, 0 °C and 28 °C. At the time point of 5 minutes, it is noticeable that the response at cold temperatures, in a genetic expression level is very low. In contrast with the response at 28° C on the same time

point, it is observed that almost 100 genes are differentially expressed. It is noticeable that the number of genes expressed shows an increase in relation to time and temperature. We can see that at 0 °C a really high number of expressed genes at the time point of 185 minutes, compared to the first time point mentioned before. The growth curve can give us some insight into these results. The exponential growth phase differs due to time as by the temperature. *Lactococcus piscium* in a cold environment has probably a slower response to its surroundings, which can possibly explain the big difference in number of differentially expressed genes, by slowing down the time it takes for the exponential growth to achieve the log phase.

The cells of all organisms have the property to respond to changes in the environment, with changes in the expression of a specific set of genes. The environmental stimulus can affect the structure and function of cellular molecules and organelles. These target molecules and organelles function as stimulus sensors, since when modified they generate signals that pass directly, or through one or more transducers, to the regulator to produce specific changes in gene expression. *Lactococcus piscium* has a total of 2403 genes, which have different types and known functions, and we can see a variety in the differentially expressed genes obtained in this study.

Temperature Specific Proteins

Known heat shock related genes CEN28048.1 (Heat-inducible transcriptional repressor HrcA) and CEN28049.1 (Nucleotide exchange factor GrpE) were observed in these 2 temperatures. At 5 minutes, I could observe an upregulation at 0 °C, while a downregulation at 185 minutes is observed, this could be an adaptation response to a sudden temperature change (Appendix 1). In contrast, at 28 °C, we observe an upregulation of said gene at both 5 and 35 minutes, GrpE is also observed at 185 minutes (Appendix 2).

Chaperone genes like CEN28050.1 (Molecular chaperone DnaK), CEN27326.1 (Molecular chaperone DnaJ), CEN27368.1 (Chaperone protein ClpB), CEN29376.1 (10 kDa chaperonin) presented an upregulation at all times at 28 °C (Appendix 2); with an exception of DnaJ at 185 minutes. I could observe an

upregulation of these genes 185 minutes in, with the exception of 10 kDa chaperonin, at 0 °C (Appendix 2).

One cold shock protein is observed. CEN27394.1 (Cold shock protein CspC) was only observed upregulated at 35 minutes at 0 °C. At 28 °C downregulation of this protein is observed, and expected (Appendix 1 & 2).

Stress genes up and downregulations were observed at both temperatures (Table 2):

CEN28155.1 (Universal stress protein UspA), CEN29325.1 (Universal stress protein UspA2), CEN29488.1 (General stress protein GlbB), CEN27327.1 (General stress protein CsbD) and CEN29584.1 (General stress protein 24). Regulation on stress genes in 0 °C is negative, while being the opposite at 28 °C.

Table 2. Stress specific proteins up and down regulation after temperature shock in *Lactococcus piscium*.

	0 °C (downregulation)		28 °C (up regulation)		
	35	185	5	35	185
<i>UspA1</i>	✓	✓			
<i>UspA2</i>	✓	✓			
<i>GlbB</i>		✓			
<i>Cls24</i>		✓		✓	✓
<i>CsbD</i>	✓	✓	✓		

Cell Adaptation

Certain surface proteins are observed. CEN27220.1 (Extracellular transglycosylase) is observed at 0 °C (35 and 185 minutes) and 28 °C (5 minutes), up and downregulated, respectively. Properties of this protein involve antimicrobial resistance (Appendix 1 & 2).

Two cell surface proteins of the Csc family are observed at 0 °C, CEN27574.1 (35 minutes, downregulated) and CEN27577.1 (185 minutes, upregulated). Known function is as a source for carbon acquisition. The exponential growth related with time might explain the upregulation at the last time point (Table 3).

Transport proteins are noticeable more upregulated at colder temperature, reaching a number of more than 60 upregulated transport genes at 185 minutes, compared to 28 °C, that only present 20. Its correlation can be pointed at the contrast in growth phase the organism has at both temperatures, at the same time point. Additionally, at 0 °C, the downregulation of ATP synthase genes (atpA, atpB, atpC, atpD, atpF, atpG, atpH) is noticeable at both 35- and 185-minutes time points (Table 3).

Cell division genes are also related to growth and time. We can observe an upregulation of one cell division related proteins at 0 °C, at a time point of 35 minutes, compared to 185 minutes, that presents an upregulation of 8 cell division related proteins (Table 3).

Table 3. Specific cell division proteins up regulation at 0 °C in *Lactococcus piscium*.

0 °C		
	35 minutes	185 minutes
<i>Cell division-specific peptidoglycan biosynthesis regulator FtsW</i>		✓
<i>Cell division transporter ATP-binding protein FtsE</i>		✓
<i>Cell division initiation protein DivIVA</i>		✓
<i>Cell division regulator GpsB</i>		✓
<i>Cell division regulator GpsB</i>		✓
<i>Cell division protein FtsK</i>		✓
<i>Cell division protein FtsX</i>		✓
<i>Cell division protein DivIC</i>		✓

<i>Cell division protein FtsL</i>	✓	✓
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Upregulation of cell surface proteins CEN27569.1 (35 minutes) and CEN27572.1 (185 minutes) is observed at 28 °C (Appendix 2). Known function in other bacteria is of adherence for colonization, what might be a cellular interactivity defense mechanism for survival. A protein with a similar function, CEN27642.1 (Aggregation promoting factor), is upregulated at 0 °C, after 185 minutes. It is also essential for maintenance of cell shape. Furthermore, CEN29228.1 (Rod shape-determining protein MreD) is observed upregulated at 0 °C, at 185 minutes time point (Appendix 1).

Different cell wall related proteins are also observed. At 0 °C, CEN27302.1 (Putative cell wall hydrolase), CEN29387.1 (Cell wall biogenesis glycosyltransferase), CEN27296.1 (Glycosyltransferase involved in cell wall biosynthesis), and CEN27733.1 (LPXTG-domain-containing protein cell wall anchor domain) are upregulated at 185 minutes. CEN28925.1 (Cell wall metabolism sensor histidine kinase VicK) is downregulated at the same time point (Appendix 1). At 28° C, upregulation of CEN28790.1 (Two-component response regulator (YvqE) responding to cell wall stress) and CEN28345.1 (Putative cell wall surface) is observed at 5 minutes, while CEN27733.1 (LPXTG-domain-containing protein cell wall anchor domain), and CEN28345.1 (Putative cell wall surface) is observed at 185 minutes. CEN27296.1 (Glycosyltransferase involved in cell wall biosynthesis) is downregulated at 5 minutes (Appendix 2).

As we can see, regulation at 0 °C can be correlated to cell wall biosynthesis and other metabolic processes, while at 28 °C is observed as a response to stress, probably linked to cell wall integrity.

Adaptation of RNA Metabolism

Adaptation in terms of RNA is more prominent at cold temperatures. Low temperature can produce stabilization of the secondary structures of nucleic acids and damage transcription. One protein that we already mentioned before, CEN27394.1 (Cold shock

protein CspC), upregulated at 0 °C, at the 35- minute time point, is essential in RNA degradation and translation, due its function in prevention of formation of secondary structures (Appendix 1).

DEAD box proteins have been found to be required in cellular processes such as RNA metabolism, including nuclear transcription, translation, and RNA degradation. CEN27648.1 (DEAD-box ATP-dependent RNA helicase CshA), and CEN27700.1 (DEAD-box ATP-dependent RNA helicase CshB) are observed upregulated at both 35 and 185 minutes time points, at 0 °C. CshA can be observed downregulated at 28 °C, at 5 and 35 minutes time points (Appendix 1 & 2).

Several other RNA related proteins can be observed

A result of a temperature downshift is a general translation block. We can observed an adaptation to prevent damage with upregulation of genes like CEN27470.1 (Translation initiation factor IF-1), CEN28088.1 (Translation initiation factor IF-3), CEN28666.1 (Peptide chain release factor 2), CEN27643.1 (Peptide chain release factor 3), CEN29413.1 (Elongation factor G, mitochondrial), and CEN29402.1 (Elongation factor Ts), all present at 185 minutes (Appendix 1 & 2).

Degradation and stabilization related genes are observed (Table 4). Upregulation of RNases is observed at 0 °C. Additionally, upregulation of a sizable amount of methyltransferase genes was observed.

Transcription initiation promoter elements are upregulated. CEN28379.1 (Sigma-70 region 2), at 0 °C, and CEN28171.1 (RNA polymerase sigma factor, sigma-70 family), at 28 °C, are observed both at 185 minutes (Appendix 1 & 2).

Table 4. RNA degradation and stabilization proteins up regulation at 0 °C in *Lactococcus piscium*.

0 °C		
	Ribonuclease	Methyltransferase

35 mins	Ribonuclease Z	Malate dehydrogenase (Oxaloacetate-decarboxylating), Malolactic enzyme
	Ribonuclease J	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
		Ribosomal RNA small subunit methyltransferase D
		tRNA (guanine-N(7)-)-methyltransferase
185 mins	Ribonuclease Z	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
	Ribonuclease Y	Ribosomal RNA small subunit methyltransferase D
	Ribonuclease J	Ribosomal RNA small subunit methyltransferase G
	Ribonuclease R	S-adenosylmethionine-dependent methyltransferase YqeM
	Ribonuclease HIII	Type I restriction-modification system DNA-methyltransferase subunit M
	Ribonuclease III	23S rRNA (Uracil-5-)-methyltransferase RumA
	Ribonuclease M5	Ribosomal RNA large subunit methyltransferase L
	Ribonuclease P protein component	LSU methyltransferase RlmI
		16S rRNA methyltransferase
		DNA-3-methyladenine glycosylase I
		Phosphomethylpyrimidine kinase
		Methylated-DNA—protein-cysteine methyltransferase
		Class I SAM-dependent methyltransferase
		Cysteine methyltransferase

Metabolic Response to Temperature Stress

Lactococcus piscium has the essential machinery to survive against different types of environmental stress. As we have observed, the MKFS47 strain is extremely sensitive to a sudden change in temperature when it is in exponential growth. (Table 5).

Cold shock upregulation is observed after 35 minutes, which could indicate that the metabolic response at cold temperatures is related to low growth rate. We can infer this due to growth related pathways being highly upregulated in comparison to others, like biosynthesis of amino acids, and energy consumption. Downregulation can be observed in fermentative processes, energy-related pathways, especially in energy production. We can also see upregulation of cell wall biogenesis and tRNA modification pathways at 185 minutes, as a possibly adaptation response for the cold.

The culture condition changes at 28 °C. Downregulation of pathways of biosynthesis of amino acids is observed. Upregulation of pathways of heat stress genes related to its adaptation and energy conservation are upregulated. For instance, lipid metabolism, due that under high temperatures permeability of biological membranes increases with temperature. The microbiological culture changes in the stationary phase that we can observe are changes in further to carbon famished conditions, as the cell utilizes glucose to produce different sources for energy production.

The full list of metabolism pathways that were seen in this study can be observed in the Appendix 3.

Table 5. Metabolic response of *Lactococcus piscium* after temperature shock.

0 °C					185 °C					
	35 min		185 min		5 min		35 min		185 min	
Pathway	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
aa Biosynthesis	✓	✓	✓	✓	✓	✓		✓		✓

<i>Amino Sugar Metabolism</i>	✓	✓	✓				✓	✓		✓
<i>Carbohydrate Biosynthesis</i>		✓		✓						
<i>Carbohydrate Degradation</i>	✓			✓		✓				
<i>Carbohydrate Metabolism</i>	✓	✓								
<i>Cell Wall Biogenesis</i>			✓							
<i>Cofactor Biosynthesis</i>		✓		✓						✓
<i>Glycan Biosynthesis</i>		✓		✓						
<i>Isoprenoid Biosynthesis</i>										
<i>Lipid Metabolism</i>				✓			✓	✓	✓	
<i>Metabolic Intermediate Metabolism</i>										
<i>Nucleotide Sugar Biosynthesis</i>	✓	✓	✓				✓	✓	✓	
<i>Polyol Metabolism</i>	✓	✓	✓		✓		✓		✓	
<i>Protein Modification</i>					✓					
<i>Purine Metabolism</i>		✓	✓					✓		✓
<i>Pyridime Metabolism</i>		✓		✓		✓				
<i>tRNA Modification</i>			✓							

Discussion

The metabolism responses to cold and warm environments are incredibly well oil machines that have develop through evolution that can be seen in both microbes and more complex organisms. The experiments carried out allow us to understand how these reactions are organized at the genomic level, unraveling the work of *Lactococcus piscium*, especially at the temperatures that are important in the food industry.

Genetic Expression After Temperature Shock

There has been extensive research to understand the ability of LAB to survive and replicate at cold temperatures [32, 33], and the effects of their growth to the environment, for food preservation purposes [34, 35]. The ability to react to environment changes is critical for survival and it is well known that LAB has defense mechanisms against stress that allow them to withstand harsh environment changes, such as temperature changes [36, 37]. With the experiments in this study and the measure of gene expression, we can see a probable relation between growth and the number of differentially expressed genes in this temperature shock experiment. *L. piscium* still presented an exponential growth after the aliquot preparation for the temperature experiment, which might be the reason for a high number of expressed genes, not only at 28 °C.

Temperature Specific Proteins

Cold Shock Protein (CspA) presented an upregulation at cold temperatures. This protein is known as a marker for cold adaptation and it was originally found as the major cold-shock protein in *E. coli* [38]. This gene was only upregulated at the time point of 185 minutes, which could possibly indicate that there is a time frame for reaction related to adaptation to cold.

Alongside CspA, CshA and CshB genes, also known as DEAD-box RNA helicase genes, were present with an extraordinarily high upregulation at 0 °C. These genes

are known for their role in cold adaptation [39], helping with the degradation and unwinding of RNA, and ribosome biogenesis at cold temperatures.

Lc. piscium also has some known heat shock genes, and all of them present an upregulation after heat shock. The main role of these genes is to maintain the proper protein-folding mechanism in cells [40]. Heat shock adaptation mechanisms seem to be only noticeable by observing the response of known heat genes and the absence of cold related stress genes. Genes like UspA, UspA2, GlbB, CsbD and General stress protein 24, could possibly give an insight of a link to a more specific function of these genes to a hot shock stress.

Upregulation of different sensor genes at different temperatures is known to be related to temperature mechanisms for sensing [41]. At 0 °C, we can observe the upregulation of the sensor histidine kinase gene (CEN28366.1) at the 185 minutes time point. In contrast, heat shock response can be observed already at 5 minutes time point. Sensor histidine kinase (CEN28791.1) could possibly be a heat sensing gene. This could indicate a probable affinity for certain temperatures and a possible need for other mechanisms to trigger these genes as part of an adaptation process.

Cell Adaptation

It has been observed in nature that bacteria can survive for long periods in a stationary phase. Some species of bacteria, like *E. coli*, changes its morphology and physiology to withstand stress [42]. The upregulation of cell shape related genes at colder temperatures it is most likely due these changes, in addition to disruption on homeostasis, triggering an adaptation mechanism. *L. piscium* is a psychotropic organism, which allows growth at colder temperatures, which is why an upregulation of some cell division genes at lower temperatures is no surprise, although we can observe in the growth curve, growth has a slower rate compared to 28 °C. In correlation with the growth curve, it is more than expected a down regulation of energy production genes to present a down regulation at temperatures below the control sample (25 °C).

In addition, we can observe the upregulation of exopolysaccharide genes that are associated with food altering abilities [43]. EPS is not utilized by the bacteria that generate it, which can create an issue on frozen food preservation, in this case, at 0 °C, where an upregulation is noticeable.

Adaptation of RNA Metabolism

There is an evident upregulation of numerous ribonuclease genes at 0 °C. Low temperatures create a stabilization of secondary structure of nucleic acids, so the relationship between RNase activity in cold adaptation, probably involves a defense mechanism against RNA degradation and translation errors. Different RNA methyltransferase genes were also upregulated at 0 °C, which, as I mentioned before, probably involves a mechanism against RNA degradation and translation errors [44-46].

Metabolic Response to Temperature Stress

Over the last decade, our understanding of the value of metabolic changes to unfavorable growth conditions has increased considerably. Normal stress tolerance is a very complex mechanism involving multiple metabolic pathways [47].

At cold temperatures, it is observed that *Lc. piscium* adapts by changing cell membrane and cell wall accordingly to changes of its environment. We observe an upregulation of tRNA processing pathway, to protect the integrity and activity of RNA [44-46]. In addition, we observe downregulation in energy related pathways, like UMP biosynthesis, and processes like fermentation and carbohydrates degradation. This is expected, as we can see a lower growth rate at colder temperatures, therefore less energy is required [48].

In contrast, at 28 °C we can observe an upregulation of stress gene response. Pathways like glycerol degradation and fatty acid and lipids degradation are upregulated, this could be related to a response mechanism of the cell membrane to warm temperatures [49]. We can also observe downregulation on pathways of amino

acids biosynthesis and amine and polyamine degradation, which it is in relationship to the lesser growth rate and the diminution of fermentative processes [50].

Conclusion

The study of the genetic response to temperature stress helps us to understand the characteristics of the sensors, signals and cellular functions of the main heat and cold stress proteins; also, to obtain an overview of the set of mechanisms that regulate the versatility of an organism for its adaptation.

Thermal shock, especially cold shock, has an important impact on bacterial growth, influencing fermentative processes, ribosome synthesis, cell wall changes, protein synthesis, and the consumption of solutes. In this analysis, *Lactococcus piscium* has been studied in a temperature contrast of 0 °C and 28 °C, providing an idea about the adaptation response in terms of its growth. A number of important genes were identified, which may be linked to the survival of this LAB, under controlled stress conditions. In this study, it was possible to observe how the abrupt change in temperature causes global alterations in genetic expression, which may result in physiological changes. This transcriptomic analysis managed to identify different genes that may have a direct relationship with a temperature adaptation response, demonstrating their effects on different metabolic pathways and the versatility of this organism to acclimatize to different environments.

Future studies should focus on analyzing the regulation and function of possible new genes for the response to heat stress, the regulation of heat stress in the response to stimuli that partially induce it, the participation of the regulation response in the induction of other types of regulation within the cell, the participation of other molecules and cellular structures in signal generation, the effect of heat stress on cell metabolism, respiration, DNA replication and cell division, as well as the specific function of proteins related to heat stress in these events.

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Diagrams made in Chemix (<https://chemix.org>)

References:

1. Bintsis, T., *Lactic acid bacteria: their applications in foods*. J Bacteriol Mycol, 2018. **6**(2): p. 89-94.
2. Carr, F.J., D. Chill, and N. Maida, *The lactic acid bacteria: a literature survey*. Critical reviews in microbiology, 2002. **28**(4): p. 281-370.
3. Khalid, K., *An overview of lactic acid bacteria*. International Journal of Biosciences, 2011. **1**(3): p. 1-13.
4. Garrity, G.M., *Bergey's manual of systematic bacteriology: Volume one: the Archaea and the deeply branching and phototrophic bacteria*. 2012: Springer Science & Business Media.
5. Stiles, M.E., *Biopreservation by lactic acid bacteria*. Antonie van leeuwenhoek, 1996. **70**(2-4): p. 331-345.
6. Kelly, W.J., L.J. Ward, and S.C. Leahy, *Chromosomal diversity in Lactococcus lactis and the origin of dairy starter cultures*. Genome biology and evolution, 2010. **2**: p. 729-744.
7. Daniel, C., et al., *Protection against Yersinia pseudotuberculosis infection conferred by a Lactococcus lactis mucosal delivery vector secreting LcrV*. Vaccine, 2009. **27**(8): p. 1141-1144.
8. Singh, V.P., *Recent approaches in food bio-preservation-a review*. Open veterinary journal, 2018. **8**(1): p. 104-111.
9. Orla-Jensen, S., *The lactic acid bacteria, Copenhagen*. Andr Fred Host and Sons imp, 1919.
10. Konigs, W., et al., *Lactic acid bacteria: the bug of the new millennium*. Curr Opin Microbiol, 2000. **3**: p. 276-282.
11. Grattepanche, F., et al., *Recent developments in cheese cultures with protective and probiotic functionalities*. Dairy Science and Technology, 2008. **88**(4-5): p. 421-444.
12. Matamoros, S., et al., *Selection and evaluation of seafood-borne psychrotrophic lactic acid bacteria as inhibitors of pathogenic and spoilage bacteria*. Food Microbiology, 2009. **26**(6): p. 638-644.
13. Fall, P.A., et al., *Inhibition of Brochothrix thermosphacta and sensory improvement of tropical peeled cooked shrimp by Lactococcus piscium CNCM I-4031*. Letters in applied microbiology, 2010. **50**(4): p. 357-361.

14. Remenant, B., et al., *Bacterial spoilers of food: behavior, fitness and functional properties*. Food microbiology, 2015. **45**: p. 45-53.
15. Teuber, M., *The genus Lactococcus*, in *The genera of lactic acid bacteria*. 1995, Springer. p. 173-234.
16. Pothakos, V., et al., *Psychrotrophic members of Leuconostoc gasicomitatum, Leuconostoc gelidum and Lactococcus piscium dominate at the end of shelf-life in packaged and chilled-stored food products in Belgium*. Food microbiology, 2014. **39**: p. 61-67.
17. Barakat, R., M. Griffiths, and L. Harris, *Isolation and characterization of Carnobacterium, Lactococcus, and Enterococcus spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat*. International journal of food microbiology, 2000. **62**(1-2): p. 83-94.
18. Sakala, R., et al., *Isolation and characterization of Lactococcus piscium strains from vacuum-packaged refrigerated beef*. Journal of applied microbiology, 2002. **92**(1): p. 173-179.
19. Tsakalidou, E. and K. Papadimitriou, *Stress responses of lactic acid bacteria*. 2011: Springer Science & Business Media.
20. Fu, B. and T.P. Labuza, *Shelf-life testing: procedures and prediction methods, in Quality in frozen food*. 1997, Springer. p. 377-415.
21. Kilcast, D. and P. Subramaniam, *The stability and shelf-life of food*. 2000.
22. Sathe, S., et al., *Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables*. Journal of Applied Microbiology, 2007. **103**(6): p. 2622-2628.
23. Leroi, F., et al., *Effect of inoculation with lactic acid bacteria on extending the shelf-life of vacuum-packed cold smoked salmon*. International journal of food science & technology, 1996. **31**(6): p. 497-504.
24. Al-Kadamany, E., et al., *Estimation of shelf-life of concentrated yogurt by monitoring selected microbiological and physicochemical changes during storage*. LWT-Food Science and Technology, 2003. **36**(4): p. 407-414.
25. Gulmez, M., N. Oral, and L. Vatansever, *The effect of water extract of sumac (Rhus coriaria L.) and lactic acid on decontamination and shelf life of raw broiler wings*. Poultry science, 2006. **85**(8): p. 1466-1471.
26. Metin, S., et al., *Extension of shelf-life of chub mackerel (Scomber japonicus Houttuyn 1780) treated with lactic acid*. European Food Research and Technology, 2001. **213**(3): p. 174-177.
27. Gould, G.W., *Methods for preservation and extension of shelf life*. International journal of food microbiology, 1996. **33**(1): p. 51-64.
28. Williams, A., J. Fryer, and M. Collins, *Lactococcus piscium sp. nov. a new Lactococcus species from salmonid fish*. FEMS Microbiology Letters, 1990. **68**(1-2): p. 109-113.
29. Garnier, M., et al., *Adaptation to cold and proteomic responses of the psychrotrophic biopreservative Lactococcus piscium strain CNCM I-4031*. Applied and environmental microbiology, 2010. **76**(24): p. 8011-8018.
30. Pothakos, V., et al., *Monitoring psychrotrophic lactic acid bacteria contamination in a ready-to-eat vegetable salad production environment*. International journal of food microbiology, 2014. **185**: p. 7-16.
31. Rahkila, R., et al., *Characterization and evaluation of the spoilage potential of Lactococcus piscium isolates from modified atmosphere packaged meat*. International journal of food microbiology, 2012. **156**(1): p. 50-59.

32. Tsvetkov, T. and I. Shishkova, *Studies on the effects of low temperatures on lactic acid bacteria*. Cryobiology, 1982. **19**(2): p. 211-214.
33. Yang, E., et al., *Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria*. AMB express, 2018. **8**(1): p. 10.
34. Stannard, C., A. Williams, and P. Gibbs, *Temperature/growth relationships for psychrotrophic food-spoilage bacteria*. Food Microbiology, 1985. **2**(2): p. 115-122.
35. Cayré, M.a.E., G. Vignolo, and O. Garro, *Modeling lactic acid bacteria growth in vacuum-packaged cooked meat emulsions stored at three temperatures*. Food Microbiology, 2003. **20**(5): p. 561-566.
36. Van De Guchte, M., et al., *Stress responses in lactic acid bacteria*. Antonie Van Leeuwenhoek, 2002. **82**(1-4): p. 187-216.
37. Papadimitriou, K., et al., *Stress physiology of lactic acid bacteria*. Microbiology and Molecular Biology Reviews, 2016. **80**(3): p. 837-890.
38. Yamanaka, K., L. Fang, and M. Inouye, *The CspA family in Escherichia coli: multiple gene duplication for stress adaptation*. Molecular microbiology, 1998. **27**(2): p. 247-255.
39. Hunger, K., et al., *Cold-induced putative DEAD box RNA helicases CshA and CshB are essential for cold adaptation and interact with cold shock protein B in Bacillus subtilis*. Journal of bacteriology, 2006. **188**(1): p. 240-248.
40. Mogk, A., et al., *Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB*. The EMBO journal, 1999. **18**(24): p. 6934-6949.
41. Abriata, L.A., et al., *Signal sensing and transduction by histidine kinases as unveiled through studies on a temperature sensor*. Accounts of chemical research, 2017. **50**(6): p. 1359-1366.
42. Ishihama, A., *Adaptation of gene expression in stationary phase bacteria*. Current opinion in genetics & development, 1997. **7**(5): p. 582-588.
43. Duboc, P. and B. Mollet, *Applications of exopolysaccharides in the dairy industry*. International Dairy Journal, 2001. **11**(9): p. 759-768.
44. Puri, P., et al., *Systematic identification of tRNAome and its dynamics in Lactococcus lactis*. Molecular microbiology, 2014. **93**(5): p. 944-956.
45. Hori, H., R. Yamagami, and C. Tomikawa, *Regulation of protein synthesis via the network between modified nucleotides in tRNA and tRNA modification enzymes in Thermus thermophilus, a thermophilic eubacterium*, in *Modified Nucleic Acids in Biology and Medicine*. 2016, Springer. p. 73-89.
46. Hori, H., *Regulatory factors for tRNA modifications in extreme-thermophilic bacterium Thermus thermophilus*. Frontiers in genetics, 2019. **10**: p. 204.
47. Chowdhury, R., G.K. Sahu, and J. Das, *Stress response in pathogenic bacteria*. Journal of Biosciences, 1996. **21**(2): p. 149-160.
48. Liu, S., et al., *Cold-stress response of probiotic Lactobacillus plantarum K25 by iTRAQ proteomic analysis*. Journal of microbiology and biotechnology, 2020. **30**(2): p. 187-195.
49. López-Lara, I.M. and O. Geiger, *Bacterial lipid diversity*. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 2017. **1862**(11): p. 1287-1299.
50. Fernandez, M. and M. Zuniga, *Amino acid catabolic pathways of lactic acid bacteria*. Critical reviews in microbiology, 2006. **32**(3): p. 155-183.

Appendix 1:

Up and down regulated genes of *L. piscium* at 0 °C

Up regulation at 5 minutes

- CEN28050.1 Molecular chaperone DnaK
- CEN28049.1 Nucleotide exchange factor GrpE
- CEN28048.1 Heat-inducible transcriptional repressor HrcA

Down regulation at 5 minutes

-

Up regulation at 35 minutes

- CEN27866.1 Malate dehydrogenase (Oxaloacetate-decarboxylating), Malolactic enzyme
- CEN27865.1 Citrate-sodium symporter
- CEN27482.1 Ketol-acid reductoisomerase
- CEN28820.1 DNA primase
- CEN27648.1 DEAD-box ATP-dependent RNA helicase CshA
- CEN28949.1 Ribonuclease Z
- CEN28947.1 Single-stranded-DNA-specific exonuclease RecJ
- CEN28948.1 Short-chain dehydrogenase
- CEN28508.1 NADH-peroxiredoxin reductase
- CEN28143.1 Penicillin-binding protein 2X PbpX
- CEN28923.1 Glutamate and aspartate transporter subunit ATP-binding component of ABC superfamily
- CEN28217.1 Ribonuclease J
- CEN28531.1 Citrate synthase
- CEN28144.1 Phospho-N-acetylmuramoyl-pentapeptide-transferase
- CEN29460.1 Multidrug and toxin extrusion (MATE) family efflux pump YdhE/NorM, homolog
- CEN28263.1 30S ribosomal protein S16
- CEN27320.1 Polysaccharide biosynthesis export protein [*Lactococcus piscium* MKFS47]
- CEN27295.1 Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA
- CEN29008.1 Primosomal protein N'
- CEN27294.1 Oligopeptide transport system permease protein AppB
- CEN27484.1 Transcriptional regulator OrfX
- CEN29278.1 Gliding motility-associated protein GldE, gldE
- CEN27215.1 tRNA(Ile)-lysine synthase
- CEN29384.1 Transporter

CEN28921.1	Glutamine transport system permease protein glnP
CEN28841.1	Replicative DNA helicase loader DnaB
CEN29487.1	Rhodanese-related sulfurtransferase
pseudogene	pseudogene
CEN27317.1	EpsH
CEN28509.1	Alkyl hydroperoxide reductase subunit C AhpC
CEN27700.1	DEAD-box ATP-dependent RNA helicase CshB
CEN27321.1	Nucleotide sugar dehydrogenase
CEN29392.1	Glycosyltransferase RgpE
CEN28946.1	Adenine phosphoribosyltransferase
CEN27392.1	D-methionine-binding lipoprotein MetQ
CEN28850.1	Aspartokinase
CEN28920.1	Amino acid ABC superfamily ATP binding cassette transporter, membrane protein
CEN28397.1	Site-specific tyrosine recombinase
CEN28212.1	Amino acid permease-associated region
CEN27839.1	Glycine betaine ABC transport system ATP-binding protein OpuAA
CEN28851.1	Haloacid dehalogenase
CEN28086.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
CEN28431.1	ABC-type transport system, multidrug-family ATP-binding/permease protein
CEN27318.1	Glycosyltransferase family protein [Lactococcus piscium MKFS47]
CEN27322.1	Exopolysaccharide biosynthesis protein EpsL
CEN28897.1	DNA-dependent RNA polymerase auxiliary subunit epsilon
CEN27803.1	Uncharacterized protein
CEN28365.1	Transcriptional regulatory protein
CEN28822.1	Major facilitator superfamily transporter
CEN29377.1	Uncharacterized protein
CEN29410.1	Ribosomal RNA small subunit methyltransferase D
CEN27293.1	Oligopeptide transport system permease protein AppC or OppC
CEN27391.1	ABC transporter permease subunit
CEN27612.1	Multidrug transport ABC transporter ATPase component
CEN29200.1	Ammonium transporter
CEN27220.1	Extracellular transglycosylase
CEN27319.1	Glycosyl transferase
CEN27314.1	Glycosyltransferase in exopolysaccharide biosynthesis
CEN28608.1	Uncharacterized protein
CEN27932.1	Predicted permease duf318
CEN29612.1	Mercuric resistance operon regulatory protein MerR
CEN28970.1	N(5)-(Carboxyethyl)ornithine synthase
CEN27315.1	Capsule biosynthesis glucosyltransferase
CEN27800.1	Negative regulator of toxin gene expression
CEN28922.1	Glutamine transport glutamine-binding protein GlnH

CEN27605.1	RibD C-terminal domain
CEN28843.1	tRNA (guanine-N(7)-)-methyltransferase
CEN28366.1	Two component sensor transduction histidine kinase
CEN27715.1	Hydroxyacylglutathione hydrolase
CEN28761.1	UDP-N-acetyl glucosamine-2-epimerase
CEN28650.1	Putative aldouronate transport system substrate-binding protein
CEN28591.1	Uncharacterized protein
CEN27316.1	Uncharacterized protein
CEN27292.1	Oligopeptide/dipeptide uptake family ABC transporter, ATP-binding protein
CEN27291.1	Oligopeptide ABC transporter ATP-binding protein OppF
CEN28361.1	Alpha-glycerophosphate oxidase
CEN27931.1	Putative HTH-type transcriptional repressor AseR
CEN28630.1	Putative HTH-type transcriptional regulator YdgJ
CEN29172.1	Uncharacterized protein
CEN27802.1	Uncharacterized protein
CEN27867.1	Malolactic fermentation system transcriptional regulator MleR
CEN28401.1	Transglutaminase
CEN27313.1	UDP-N-acetylglucosamine--LPS N-acetylglucosamine transferase
CEN27714.1	Lactoylglutathione lyase
CEN27642.1	Aggregation promoting factor
CEN28806.1	Protein of unknown function DUF1310
CEN29117.1	Outer membrane lipoprotein [Lactococcus piscium MKFS47]
CEN27390.1	ABC-type metal ion transport system, ATPase component
CEN28186.1	Uncharacterized protein
CEN27492.1	Oligopeptide transporter YclF
CEN28079.1	Putative membrane protein
CEN29417.1	pseudogene
CEN28876.1	Uncharacterized protein YshB
CEN28142.1	Cell division protein FtsL
CEN28750.1	Predicted hydrolases of the HAD superfamily
CEN28874.1	Nanthine-uracil permease
CEN29409.1	Pantetheine-phosphate adenylyltransferase
CEN27253.1	Transport permease protein
CEN28875.1	Xanthine phosphoribosyltransferase
CEN27586.1	Malolactic fermentation system transcriptional regulator MleR
CEN28977.1	Adenine-specific DNA methyltransferase
CEN28515.1	Two-component system KDP operon response regulator KdpE
CEN29582.1	Uncharacterized protein
CEN27981.1	GNAT family N-acetyltransferase
CEN28198.1	Hyaluronan synthase
CEN29557.1	Peptide transporter
CEN28083.1	Anticodon nuclease PrrC

CEN28387.1	CBS domain-containing protein YkuL
CEN27583.1	HTH domain
CEN27840.1	Glycine betaine ABC transporter permease and substrate-binding component
CEN27723.1	Uncharacterized protein
CEN28988.1	Uncharacterized protein
CEN29088.1	Uncharacterized protein YdbD
CEN29372.1	Uncharacterized protein
CEN28719.1	Integral membrane protein
CEN28686.1	Phosphotransferase system PTS sorbose-specific IIC subunit
CEN28647.1	DUF624-containing protein
CEN27252.1	Uncharacterized protein
CEN27647.1	ABC-type multidrug transport system permease component
CEN28590.1	Bacteriocin

Down regulation at 35 minutes

CEN28220.1	Aspartate carbamoyltransferase
CEN27424.1	Cyclopropane-fatty-acyl-phospholipid synthase
CEN29325.1	Universal stress protein UspA
CEN28225.1	Dihydroorotate dehydrogenase B (NAD(+)), electron transfer subunit
CEN28221.1	Carbamoyl-phosphate synthase small subunit
CEN28218.1	Bifunctional protein PyrR
CEN29012.1	S-adenosylmethionine synthase
CEN28058.1	ATP synthase subunit c
CEN28041.1	Bile salt hydrolase
CEN28060.1	ATP synthase subunit b
CEN28227.1	Orotidine 5'-phosphate decarboxylase
CEN27494.1	Beta-ketoacyl-acyl carrier protein reductase
CEN29599.1	Acetoin dehydrogenase
CEN27357.1	Phosphoglycerate kinase
CEN29439.1	Serine protease HtrA
CEN29053.1	C3-degrading proteinase
CEN28059.1	ATP synthase subunit a
CEN29594.1	Predicted oxidoreductase Myosin-crossreactive antigen ortholog
CEN28155.1	Universal stress protein UspA
CEN29021.1	Flavodoxin
CEN29206.1	Manganese-dependent inorganic pyrophosphatase
CEN28992.1	Methyl-accepting chemotaxis-like domain containing protein
CEN29223.1	Histidine-containing phosphocarrier protein of the PTS
CEN28449.1	dTDP-4-keto-6-deoxy-D-glucose epimerase
CEN28928.1	Fructose-bisphosphate aldolase
CEN29576.1	Putative iron export ATP-binding protein FetA

CEN28884.1	Glucose-6-phosphate isomerase
CEN29382.1	Glutamyl aminopeptidase
CEN29577.1	Predicted membrane protein of the YbbM family
CEN27673.1	ATP-dependent Clp protease proteolytic subunit
CEN28488.1	UDP-glucose 4-epimerase GalE
CEN29429.1	Inosine-5'-monophosphate dehydrogenase
CEN28240.1	Redox-sensing transcriptional repressor Rex
CEN28219.1	Uracil permease PyrP
CEN29230.1	Hydrolase acting on acid anhydrides in phosphorous-containing anhydrides
CEN28661.1	Serine--tRNA ligase
CEN29208.1	Enterocin A immunity
CEN27361.1	Catechol 2,3-dioxygenase
CEN28222.1	Carbamoyl-phosphate synthase large subunit
CEN27587.1	Enterocin A Immunity
CEN28595.1	Metal-dependent hydrolase Yhfl
CEN27980.1	Uncharacterized protein
CEN27686.1	Adenylate cyclase
CEN28357.1	Carboxy-cis,cis-muconate cyclase
CEN29449.1	ADP-ribose pyrophosphatase
CEN28226.1	Dihydroorotate dehydrogenase
CEN29090.1	Uncharacterized protein
CEN27420.1	Lactose operon transcriptional regulator
CEN28811.1	Arsenate reductase ArsC
CEN29232.1	Ribose-5-phosphate isomerase A
CEN27848.1	Hydroxyacylglutathione hydrolase
CEN29440.1	Uncharacterized protein
CEN27778.1	Cystathionine gamma-synthase and O-acetylhomoserine thiolase
CEN29132.1	Putative phage tail protein
CEN29144.1	Phage capsid family
CEN28526.1	LrgA-associated membrane protein LrgB
CEN29578.1	Uncharacterized protein
CEN28303.1	Cobalamin-independent methionine synthase II
CEN29128.1	Bacteriophage peptidoglycan hydrolase
CEN27769.1	4-oxalocrotonate tautomerase Xylose transport system permease protein xylH
CEN27903.1	Uncharacterized protein
CEN27488.1	Uncharacterized protein
CEN29235.1	L-rhamnose operon regulatory protein rhaS
CEN28271.1	Uncharacterized protein
CEN29587.1	Chlorophyll synthase ChlG, chlG
CEN28307.1	Bacteriocin immunity protein Skgl
CEN27701.1	Multiple sugar-binding transport ATP-binding protein MsmK
CEN28990.1	Signal peptide containing protein

CEN28259.1	Putative DNA topology modulation protein FlaR
CEN29575.1	Uncharacterized protein
CEN28869.1	Trehalose operon transcriptional repressor
CEN28441.1	Predicted 4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase
CEN28161.1	Predicted SAM-dependent methyltransferase
CEN29292.1	Acid-resistance membrane protein
CEN28671.1	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase DhaL
CEN28270.1	Acetyltransferases, including N-acetylases of ribosomal proteins
CEN29145.1	Phage scaffold protein
CEN27574.1	Cell surface protein, CscB family
CEN27904.1	Uncharacterized protein
CEN28935.1	Ribose 5-phosphate isomerase
CEN29489.1	Plasmid partitioning protein ParB
CEN28166.1	Uncharacterized protein
CEN27327.1	General stress protein CsbD
CEN27548.1	Phage envelope protein
CEN28911.1	HTH-type transcriptional regulator GmuR
CEN27395.1	5-formyltetrahydrofolate cyclo-ligase
CEN27588.1	Uncharacterized protein
CEN27607.1	Transcriptional regulator YeiE
CEN29459.1	Membrane protein (Modular protein)
CEN27421.1	Aldose 1-epimerase
CEN28693.1	Phosphoribosylamine--glycine ligase
CEN29221.1	Transcriptional regulators of sugar metabolism
CEN28329.1	Alpha/beta hydrolase
CEN27634.1	Membrane-associated phospholipid phosphatase
CEN28157.1	HTH-type transcriptional regulator AdhR
CEN29141.1	Uncharacterized protein

Up regulation at 185 minutes

CEN27648.1	DEAD-box ATP-dependent RNA helicase CshA
CEN27474.1	DNA-directed RNA polymerase subunit alpha
CEN28947.1	Single-stranded-DNA-specific exonuclease RecJ
CEN27470.1	Translation initiation factor IF-1
CEN27700.1	DEAD-box ATP-dependent RNA helicase CshB
CEN27475.1	50S ribosomal protein L17
CEN28948.1	Short-chain dehydrogenase
CEN29393.1	ATP-binding protein ABC transporter system for polysaccharides
CEN29415.1	30S ribosomal protein S12
CEN28397.1	Site-specific tyrosine recombinase
CEN28949.1	Ribonuclease Z
CEN28945.1	DNA-directed RNA polymerase subunit delta

CEN29011.1	Ribonuclease Y
CEN28820.1	DNA primase
CEN27482.1	Ketol-acid reductoisomerase
CEN27472.1	30S ribosomal protein S13
CEN28355.1	Zinc ABC transporter substrate-binding protein AdcA
CEN29414.1	30S ribosomal protein S7
CEN29095.1	Uncharacterized protein YuhC
CEN27302.1	Putative cell wall hydrolase [Lactococcus piscium MKFS47]
CEN29294.1	Protein RecA
CEN27447.1	30S ribosomal protein S10
CEN27471.1	50S ribosomal protein L36
CEN29556.1	Replication protein RepB
CEN28839.1	Ribosome biogenesis GTPase Der
CEN27595.1	Single-stranded DNA-binding protein 2
CEN27956.1	30S ribosomal protein S4
CEN29408.1	Endopeptidase La
CEN28946.1	Adenine phosphoribosyltransferase
CEN29228.1	Rod shape-determining protein MreD
CEN28531.1	Citrate synthase
CEN28263.1	30S ribosomal protein S16
CEN29225.1	Phospho-2-dehydro-3-deoxyheptonate aldolase
CEN29401.1	Septation ring formation regulator EzrA
CEN27266.1	ABC-F family ATPase
CEN29396.1	Rhamnosyltransferase RgpA
CEN27839.1	Glycine betaine ABC transport system ATP-binding protein OpuAA
CEN28931.1	Cell division-specific peptidoglycan biosynthesis regulator FtsW
CEN27208.1	Ribosome-binding ATPase YchF
CEN28217.1	Ribonuclease J
CEN27518.1	GTP-sensing transcriptional pleiotropic repressor CodY
CEN29388.1	Uncharacterized protein
CEN27310.1	Tyrosine protein kinase EpsB
CEN27330.1	Ribosome biogenesis GTPase YqeH
CEN28851.1	Haloacid dehalogenase
CEN27473.1	30S ribosomal protein S11
CEN28923.1	Glutamate and aspartate transporter subunit ATP-binding component of ABC superfamily
CEN27840.1	Glycine betaine ABC transporter permease and substrate-binding component
CEN28191.1	Teichoic acids export ATP-binding protein TagH
CEN29384.1	Transporter
CEN29391.1	Rhamnan synthesis protein F
CEN29413.1	Elongation factor G, mitochondrial
CEN29278.1	Gliding motility-associated protein GldE, gldE
CEN29383.1	Peptidoglycan amidohydrolase

CEN28961.1	Ribosome biogenesis GTPase YqeH
CEN28999.1	RNA-binding protein YlmH, contains S4-like domain
CEN27448.1	50S ribosomal protein L3
CEN28609.1	Small GTP-binding protein domain:GTP-binding protein TypA
CEN28193.1	Transferase for other substituted phosphate groups
CEN28029.1	Ketoacyl-ACP synthase III
CEN29392.1	Glycosyltransferase RgpE
CEN29395.1	Polysaccharide biosynthesis protein/ rhamnosyl transferase
CEN29261.1	Inositol-phosphate phosphatase
CEN29485.1	Arginine/ornithine antiporter ArcD
CEN28028.1	Transcriptional regulator of fatty acid biosynthesis FabT
CEN28628.1	ABC-type multidrug transport system, ATPase and permease components
CEN29195.1	Putative oligopeptide-binding protein AmiA
CEN27306.1	Branched-chain amino acid transport ATP-binding protein LivF
CEN28874.1	Nanthine-uracil permease
CEN27238.1	50S ribosomal protein L13
CEN29010.1	Guanylate kinase
CEN27691.1	Peptidyl-prolyl cis-trans isomerase
CEN29226.1	Preprotein translocase subunit SecA
CEN28921.1	Glutamine transport system permease protein glnP
CEN27444.1	Transcription termination/antitermination protein NusG
CEN28192.1	Teichoic acid ABC transporter permease
CEN28086.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
CEN27596.1	30S ribosomal protein S18
CEN27838.1	Transcriptional repressor BusR
CEN28828.1	Beta-glucoside operon transcriptional antiterminator
CEN27321.1	Nucleotide sugar dehydrogenase
CEN28930.1	Pyruvate carboxylase
CEN27322.1	Exopolysaccharide biosynthesis protein EpsL
CEN29258.1	Trigger factor
CEN27309.1	Polysaccharide biosynthesis protein, chain length regulator EpsA
CEN28838.1	Inner membrane protein YrbG, predicted calcium/sodium:proton antiporter
CEN29061.1	PIN/TRAM domain-containing protein
CEN28481.1	Poly(glycerophosphate chain) D-alanine transfer protein DltD
CEN27483.1	L-threonine dehydratase
CEN29394.1	Transport permease protein
CEN28655.1	Pore forming protein ebsA
CEN29410.1	Ribosomal RNA small subunit methyltransferase D
CEN28844.1	Aminoglycoside phosphotransferase
CEN27304.1	High-affinity branched-chain amino acid ABC transporter permease LivM

CEN28656.1	Hydrogen peroxide-inducible transcriptional activator
CEN29212.1	ABC transporter
CEN27694.1	Bacterial extracellular solute-binding lipoprotein, family 3
CEN27215.1	tRNA(Ile)-lysine synthase
CEN29403.1	30S ribosomal protein S2
CEN29308.1	RNA-binding protein YaaA
CEN28845.1	Putative protein Veg
CEN27657.1	DNA-directed RNA polymerase subunit beta
CEN29387.1	Cell wall biogenesis glycosyl transferase
CEN28114.1	Ribosomal protein S1 RpsA
CEN29386.1	Glycosyltransferase, TIGR04182 family
CEN27743.1	Penicillin-binding protein 1A
CEN27615.1	50S ribosomal protein L33 type 3
CEN28850.1	Aspartokinase
CEN27311.1	Tyrosine-protein phosphatase YwqE
CEN28223.1	Lead cadmium zinc and mercury transporting ATPase Copper-translocating P-type ATPase
CEN28922.1	Glutamine transport glutamine-binding protein GlnH
CEN27457.1	30S ribosomal protein S17
CEN28962.1	YqeG family HAD IIIA-type phosphatase
CEN29194.1	Oligopeptide transport system permease protein AmiC
CEN28944.1	Endolytic murein transglycosylase
CEN29418.1	Putative sugar uptake protein YxfA
CEN29389.1	O antigen biosynthesis rhamnosyltransferase RfbN
CEN28017.1	Foldase protein PrsA
CEN28665.1	Cell division transporter ATP-binding protein FtsE
CEN29236.1	ATP-dependent RNA helicase YxiN
CEN29224.1	Phospho-2-dehydro-3-deoxyheptonate aldolase
CEN27315.1	Capsule biosynthesis glucosyltransferase
CEN27219.1	Membrane associated acyltransferase
CEN28933.1	Multidrug resistance efflux pump PmrA
CEN27958.1	Uncharacterized protein
CEN27594.1	Ribosomal S6 domain containing protein
CEN29315.1	Multimodular transpeptidase-transglycosylase
CEN28735.1	Glutamine ABC transporter permease and substrate-binding components
CEN28630.1	Putative HTH-type transcriptional regulator YdgJ
CEN27932.1	Predicted permease duf318
CEN28776.1	Ribonuclease R
CEN27714.1	Lactoylglutathione lyase
CEN28666.1	Peptide chain release factor 2
CEN28920.1	Amino acid ABC superfamily ATP binding cassette transporter, membrane protein
CEN29227.1	Secreted antigen GbpB/Peptidoglycan hydrolase

CEN28395.1	Branched-chain amino acid transport system II carrier protein
CEN29200.1	Ammonium transporter
CEN28342.1	PEP-CTERM system TPR-repeat lipoprotein, prsT
CEN27441.1	Penicillin-binding protein 2A
CEN27612.1	Multidrug transport ABC transporter ATPase component
CEN29279.1	Predicted PurR-regulated permease PerM
CEN29059.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
CEN27314.1	Glycosyltransferase in exopolysaccharide biosynthesis
CEN28998.1	Cell division initiation protein DivIVA
CEN28264.1	KH domain-containing protein
CEN28054.1	Sodium ABC transporter permease
CEN27334.1	Ribosomal silencing factor RsfS
CEN27469.1	Adenylate kinase
CEN29402.1	Elongation factor Ts
CEN27394.1	Cold shock protein CspC
CEN27312.1	Undecaprenyl-phosphate galactose phosphotransferase
CEN29309.1	DNA replication and repair protein RecF
CEN28879.1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
CEN29326.1	Peptidase T
CEN29443.1	Glycosyltransferase LafA responsible for the formation of Glc-DAG
CEN28251.1	TetR/AcrR family transcriptional regulator
CEN27803.1	Uncharacterized protein
CEN27333.1	Metal dependent phosphohydrolase
CEN28284.1	Fructose-1-phosphate phosphatase YqaB
CEN29319.1	NifS/lcsS protein homolog
CEN28053.1	Putative Na(+) ABC exporter, ATP-binding subunit
CEN29126.1	Uncharacterized protein
CEN27446.1	Large-conductance mechanosensitive channel protein MscL
CEN27467.1	50S ribosomal protein L15
CEN29201.1	Phosphotransferase with a nitrogenous group as acceptor
CEN27461.1	30S ribosomal protein S14 type Z
CEN28340.1	Aromatic acid exporter family member 1
CEN28591.1	Uncharacterized protein
CEN27329.1	Predicted hydrolase of the HAD superfamily
CEN29612.1	Mercuric resistance operon regulatory protein MerR
CEN28285.1	Transposase
CEN27705.1	Undecaprenyl-phosphate alpha-N-acetylglucosaminephosphotransferase
CEN28571.1	ABC superfamily ATP binding cassette transporter, binding protein
CEN28044.1	Ribonucleotide reductase/pyruvate formate lyase family protein (UPF0210 protein)
CEN29081.1	Methionine import ATP-binding protein MetN
CEN28575.1	L-lactate dehydrogenase
CEN29008.1	Primosomal protein N'

CEN27445.1	Threonine synthase
CEN29060.1	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
CEN28089.1	50S ribosomal protein L35
CEN28088.1	Translation initiation factor IF-3
CEN29211.1	ABC-type multidrug/protein/lipid transport system, ATPase component
CEN27317.1	EpsH
CEN27640.1	Trifunctional nucleotide phosphoesterase protein YfkN
CEN29191.1	Oligopeptide ABC transporter ATP-binding protein AmiF
CEN28010.1	Glutaredoxin
CEN27643.1	Peptide chain release factor 3
CEN27450.1	50S ribosomal protein L23
CEN27650.1	Membrane protein for polysaccharide transport
CEN29193.1	Abc transporter, oligopeptide transported permease component
CEN27693.1	Arginine ABC transporter ATP-binding protein ArtP
CEN28464.1	Dihydrofolate reductase
CEN29329.1	50S ribosomal protein L21
CEN27649.1	GTPase Era
CEN28230.1	Uncharacterized protein
CEN27237.1	Ribonuclease HIII
CEN27318.1	Glycosyltransferase family protein [Lactococcus piscium MKFS47]
CEN28822.1	Major facilitator superfamily transporter
CEN29390.1	Serotype b-specific antigen synthesis gene cluster
CEN27926.1	Sodium/hydrogen antiporter
CEN29460.1	Multidrug and toxin extrusion (MATE) family efflux pump YdhE/NorM, homolog
CEN29009.1	DNA-directed RNA polymerase subunit omega
CEN29094.1	U32 family peptidase small subunit
CEN29262.1	UPF0223 protein
CEN28608.1	Uncharacterized protein
CEN27331.1	Ancient RNA-binding IF3-C fold protein
CEN27301.1	Amino acid/amide ABC transporter substrate-binding protein, HAAT family
CEN28976.1	Type II restriction endonuclease
CEN27217.1	ATP-dependent zinc metalloprotease FtsH
CEN28977.1	Adenine-specific DNA methyltransferase
CEN29583.1	Ferredoxin-NADP reductase
CEN27218.1	MORN repeat protein
CEN27332.1	Nicotinate-nicotinamide nucleotide adenyltransferase
CEN27799.1	Prophage site-specific recombinase
CEN28897.1	DNA-dependent RNA polymerase auxiliary subunit epsilon
CEN29085.1	DUF368 domain-containing protein
CEN29213.1	Transcriptional regulators
CEN27452.1	30S ribosomal protein S19

CEN27319.1	Glycosyl transferase
CEN29002.1	Pyridoxal phosphate homeostasis protein
pseudogene	pseudogene
CEN28212.1	Amino acid permease-associated region
CEN28881.1	SatA
CEN28228.1	Cell division regulator GpsB
CEN28474.1	Heptaprenyl diphosphate synthase component I YnhH
CEN29040.1	Phenazine biosynthesis protein PhzF like
CEN28476.1	Ribosomal RNA small subunit methyltransferase G
CEN27466.1	50S ribosomal protein L30
CEN29318.1	Probable tRNA sulfurtransferase
CEN28187.1	Cell division protein FtsK
CEN27335.1	S-adenosylmethionine-dependent methyltransferase YqeM
CEN28254.1	Pyruvate dehydrogenase subunit alpha
CEN28970.1	N(5)-(Carboxyethyl)ornithine synthase
CEN27438.1	Predicted membrane protein
CEN29117.1	Outer membrane lipoprotein [Lactococcus piscium MKFS47]
CEN28384.1	Glutamate racemase
CEN27651.1	30S ribosomal protein S10
CEN27658.1	DNA-directed RNA polymerase subunit beta'
CEN28188.1	DNA polymerase III delta subunit HolA
CEN29331.1	Adenylosuccinate synthetase
CEN28664.1	Cell division protein FtsX
CEN28244.1	Protein of hypothetical function DUF1054
CEN27585.1	Uncharacterized protein
CEN28341.1	UbiC transcription regulator-associated
CEN28142.1	Cell division protein FtsL
CEN28777.1	Preprotein translocase subunit SecG
CEN28653.1	Cytidylate kinase
CEN27356.1	30S ribosomal protein S21
CEN29263.1	Transcriptional regulator Spx
CEN27692.1	Putative arginine ABC transporter, permease protein ArtQ
CEN27941.1	Site-specific integrase
CEN27580.1	Glutamine ABC transporter glutamine-binding protein/permease
CEN27209.1	DUF951 domain-containing protein
CEN28213.1	Aspartate-semialdehyde dehydrogenase
CEN29045.1	Uridylate kinase
CEN28405.1	Ribosome biogenesis GTPase YlqF
CEN27220.1	Extracellular transglycosylase
CEN28252.1	Sugar phosphate phosphatase
CEN27308.1	Acyl-phosphate glycerol 3-phosphate acyltransferase
CEN29581.1	HTH-type transcriptional regulator
CEN29477.1	EcsC protein family

CEN28253.1	Phosphohydrolase
CEN28387.1	CBS domain-containing protein YkuL
CEN28747.1	Phosphoribosylglycinamide synthetase
CEN27462.1	30S ribosomal protein S8
CEN28077.1	Type I restriction-modification system DNA-methyltransferase subunit M
CEN27638.1	Phosphonate transport system permease protein phnB
CEN28027.1	Nitronate monooxygenase
CEN28852.1	P-type HAD superfamily ATPase
CEN27297.1	GTP pyrophosphokinaseppGpp synthetase I
CEN27440.1	Helix turn helix arsenical resistance operon repressor
CEN27463.1	50S ribosomal protein L6
CEN27602.1	23S rRNA (Uracil-5-)-methyltransferase RumA
CEN29409.1	Pantetheine-phosphate adenylyltransferase
CEN29180.1	Uncharacterized protein
CEN27917.1	Nucleoside diphosphates hydrolase
CEN28969.1	ATPase
CEN28180.1	Uridine kinase
CEN27305.1	High-affinity branched-chain amino acid ABC transporter ATP-binding protein LivG
CEN27957.1	Site-specific recombinase XerD
CEN28078.1	Oligopeptide ABC transporter periplasmic oligopeptide-binding protein OppA
CEN28431.1	ABC-type transport system, multidrug-family ATP-binding/permease protein
CEN27214.1	Beta-lactamase-type transpeptidase YacG
CEN27744.1	Brp/Blh family beta-carotene 15,15'-monooxygenase
CEN28138.1	tRNA-dependent lipid II-Ala--L-alanine ligase
CEN27639.1	Phosphonate ABC transporter permease protein PhnE
CEN27313.1	UDP-N-acetylglucosamine--LPS N-acetylglucosamine transferase
CEN27800.1	Negative regulator of toxin gene expression
CEN28736.1	Ectoine/hydroxyectoine ABC transporter, ATP-binding protein EhuA, ehuA
CEN29199.1	Nitrogen regulatory protein P-II for glutamine synthetase
CEN29093.1	U32 family peptidase large subunit
CEN27291.1	Oligopeptide ABC transporter ATP-binding protein OppF
CEN27396.1	GlpG protein (Membrane protein of glp regulon)
CEN27443.1	Preprotein translocase subunit SecE
CEN27295.1	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA
CEN27398.1	PTS system, cellobiose-specific IIC component
CEN27603.1	N-acetyltransferase
CEN29297.1	33 kDa chaperonin
CEN29080.1	Binding-protein-dependent transport system inner membrane component

CEN27893.1	Uncharacterized protein
CEN27742.1	Holliday junction resolvase RecU
CEN27236.1	Signal peptidase I
CEN27605.1	RibD C-terminal domain
CEN28750.1	Predicted hydrolases of the HAD superfamily
CEN27985.1	Bacteriocin export ABC transporter
CEN27642.1	Aggregation promoting factor
CEN27212.1	S1 RNA binding domain protein
CEN29299.1	Transporter
CEN27449.1	Ribosomal protein L4 (Fragment)
CEN27614.1	50S ribosomal protein L32
CEN28805.1	Ribonuclease III
CEN28479.1	D-alanyl-lipoteichoic acid biosynthesis protein DltB
CEN27216.1	Hypoxanthine-guanine phosphoribosyltransferase
CEN27222.1	Membrane-associated phospholipid phosphatase
CEN29086.1	Cys-tRNA(Pro)/Cys-tRNA(Cys) deacylase
CEN27637.1	Phosphonates import ATP-binding protein PhnC
CEN27618.1	Uncharacterized protein
CEN28257.1	Phosphoribosylformylglycinamidine cyclo-ligase
CEN28430.1	Putative ABC transporter, ATP-binding/permease protein
CEN28229.1	Ribosomal RNA large subunit methyltransferase L
CEN29399.1	dTDP-4-keto-6-deoxy-D-glucose epimerase
CEN27704.1	Negative regulator of genetic competence MecA
CEN27918.1	Uncharacterized protein
CEN29058.1	Glutamate--tRNA ligase
CEN27258.1	Transcription regulator
CEN28477.1	Teichoic acid D-Ala incorporation-associated protein DltX
CEN27931.1	Putative HTH-type transcriptional repressor AseR
CEN27535.1	50S ribosomal protein L28
CEN28224.1	N-acetyltransferase
CEN28781.1	Ectoine/hydroxyectoine ABC transporter, permease protein EhuC, ehuC
CEN28043.1	ACT domain-containing protein
CEN27320.1	Polysaccharide biosynthesis export protein [Lactococcus piscium MKFS47]
CEN27293.1	Oligopeptide transport system permease protein AppC or OppC
CEN28517.1	Pseudouridine synthase
CEN27583.1	HTH domain
CEN28255.1	Alpha-ketoacid dehydrogenase subunit beta
CEN27478.1	Dihydroxy-acid dehydratase
CEN29287.1	Glycerophosphodiester phosphodiesterase domain of glycerophosphodiester phosphodiesterase
CEN27292.1	Oligopeptide/dipeptide uptake family ABC transporter, ATP-binding protein

CEN28084.1	Type I restriction endonuclease subunit R
CEN28974.1	Putative type II restriction endonuclease
CEN29106.1	Copper homeostasis protein CutC
CEN28775.1	SsrA-binding protein SmpB
CEN27262.1	DNA repair protein RecO
CEN28473.1	Protein export element
CEN27460.1	50S ribosomal protein L5
CEN27316.1	Uncharacterized protein
CEN28090.1	50S ribosomal protein L20
CEN28834.1	RNA-binding protein YlxR
CEN28480.1	D-alanyl carrier protein
CEN28145.1	Promiscuous phosphatase
CEN27435.1	Zinc transport transcriptional regulator
CEN28700.1	Signal peptidase I
CEN28200.1	UDP-N-acetylenolpyruvoylglucosamine reductase
CEN27933.1	Small redox-active disulfide protein 2
CEN29324.1	Uncharacterized protein
CEN28439.1	30S ribosomal protein S9
CEN29092.1	Cytochrome O ubiquinol oxidase
CEN28475.1	Heptaprenyl diphosphate synthase component II HepS
CEN27210.1	RNA-binding S4 domain-containing protein
CEN28782.1	L-Cystine ABC transporter periplasmic cystine-binding protein TcyA
CEN29417.1	pseudogene
CEN29385.1	DUF2304-containing protein
CEN28979.1	ATPase V
CEN28784.1	Uncharacterized protein
CEN28080.1	Uncharacterized protein
CEN28154.1	Membrane domain of membrane-anchored glycerophosphoryl diester phosphodiesterase
CEN28748.1	Uncharacterized protein
CEN28083.1	Anticodon nuclease PrrC
CEN28461.1	Probable GTP-binding protein EngB
CEN29044.1	Ribosome-recycling factor
CEN28583.1	Uncharacterized protein
CEN28876.1	Uncharacterized protein YshB
CEN28365.1	Transcriptional regulatory protein
CEN28703.1	ABC transporter ATP-binding protein YfmM
CEN27294.1	Oligopeptide transport system permease protein AppB
CEN27296.1	Glycosyltransferase involved in cell wall biosynthesis
CEN29461.1	N-acetyltransferase
CEN29609.1	Cu(I)-responsive transcriptional regulator, cueR
CEN27703.1	Glutamate ABC transporter ATP-binding protein
CEN27723.1	Uncharacterized protein

CEN28370.1	Putative SRP operon regulator YlxM
CEN28589.1	Sugar efflux transporter for intercellular exchange
CEN27604.1	Aldose epimerase
CEN27303.1	High-affinity branched-chain amino acid transport system permease protein LivH
CEN28151.1	Low-complexity acidic protein
CEN27916.1	30S ribosomal protein S14
CEN28260.1	Fructose permease
CEN27486.1	Alkylphosphonate uptake protein in phosphonate metabolism
CEN27404.1	Oxygen-insensitive NAD(P)H nitroreductase / Dihydropteridine reductase
CEN28202.1	Uncharacterized protein
CEN28743.1	Membrane spanning protein [Lactococcus piscium MKFS47]
CEN28219.1	Uracil permease PyrP
CEN28079.1	Putative membrane protein
CEN28742.1	DMT family transporter
CEN27741.1	UPF0398 protein
CEN28296.1	LSU methyltransferase Rlml
CEN27397.1	Phosphotransferase PTS system cellobiose lichenan-specific IIA subunit
CEN27776.1	Regulatory protein, TetR
CEN28987.1	Uncharacterized protein
CEN28701.1	Uncharacterized protein YqjA
CEN28184.1	Putative Type III restriction-modification system, modification subunit
CEN28396.1	O-antigen acetylase WbiA
CEN28829.1	PTS system beta-glucoside-specific transporter subunit IIABC
CEN28956.1	ABC-type phosphate phosphonate transporter permease component
CEN27532.1	Prophage ps1 protein 20
CEN28572.1	Uncharacterized protein
CEN28629.1	Uncharacterized protein
CEN28186.1	Uncharacterized protein
CEN29478.1	Membrane protein containing DUF1211 [Lactococcus piscium MKFS47]
CEN27715.1	Hydroxyacylglutathione hydrolase
CEN28980.1	Cation (Potassium) transport associated protein
CEN28392.1	Riboflavin transporter RibU
CEN28683.1	6-phospho 3-hexuloisomerase, hxlB
CEN27254.1	Transport protein YcxC
CEN28875.1	Xanthine phosphoribosyltransferase
CEN29286.1	Membrane domain of glycerophosphoryl diester phosphodiesterase
CEN28389.1	Segregation and condensation protein A
CEN27907.1	Uncharacterized protein

CEN28401.1	Transglutaminase
CEN28339.1	30S ribosomal protein S20
CEN28299.1	Esterase
CEN27391.1	ABC transporter permease subunit
CEN28018.1	Tubby C 2
CEN28955.1	Serine/threonine protein phosphatase
CEN28185.1	Type II restriction-modification system restriction subunit
CEN28482.1	Bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase PurH
CEN29374.1	Prophage ps2 probable integrase
CEN28989.1	Uncharacterized protein
CEN28002.1	Hemolysin HyIII
CEN28425.1	Glycerol-3-phosphate acyltransferase
CEN29181.1	Site-specific tyrosine recombinase XerC
CEN29214.1	Predicted signal peptide containing peptidase
CEN28942.1	Transcriptional regulator CtsR
CEN28603.1	Uncharacterized protein
CEN28636.1	MerR family HTH transcriptional regulator
CEN29442.1	Glycosyltransferase LafB responsible for the formation of Gal-Glc- DAG
CEN29041.1	Protein of hypothetical function DUF1250
CEN28367.1	Uncharacterized protein
CEN27270.1	Type II secretion system protein GspE
CEN28640.1	Uncharacterized protein
CEN28204.1	Glycerol-3-phosphate cytidyltransferase
CEN29282.1	Ribonuclease M5
CEN28518.1	Lipoprotein signal peptidase
CEN28171.1	RNA polymerase sigma factor, sigma-70 family
CEN28619.1	DNA polymerase IV
CEN28300.1	16S rRNA methyltransferase
CEN27492.1	Oligopeptide transporter YclF
CEN27403.1	Teicoplanin resistance protein VanZ
CEN27959.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN28957.1	Phosphonate ABC transporter permease component
CEN28026.1	Dihydroorotate dehydrogenase, catalytic subunit
CEN28005.1	YfaA
CEN28196.1	Glycosyl transferase, group 2 family protein
CEN29590.1	Transcriptional regulators
CEN29243.1	Positive transcriptional regulator, MutR family
CEN29269.1	Dihydroneopterin aldolase FolB [Lactococcus piscium MKFS47]
CEN27644.1	LytTR family transcriptional regulator
CEN28958.1	Phosphate/phosphite/phosphonate ABC transporters, periplasmic binding protein

CEN27802.1	Uncharacterized protein
CEN29017.1	Queuosine ECF transporter substrate-specific component QueT
CEN27663.1	ABC transporter periplasmic protein family 3
CEN28132.1	Exodeoxyribonuclease 7 small subunit
CEN29420.1	Sugar transport protein
CEN27509.1	Ribonuclease P protein component
CEN27433.1	Acetyltransferase
CEN28448.1	Sensor histidine kinase
CEN29604.1	Cadmium resistance transcriptional regulatory protein CadC
CEN28358.1	AI-2E family transporter
CEN27826.1	FRG domain-containing protein [Lactococcus piscium MKFS47]
CEN28827.1	Multidrug transporter
CEN27269.1	Uncharacterized protein
CEN28932.1	50S ribosomal protein L33
CEN28651.1	Transcriptional regulator
CEN27259.1	GNAT family acetyltransferase
CEN27476.1	Gamma-glutamylcysteine synthetase
CEN27484.1	Transcriptional regulator OrfX
CEN29486.1	DNA-3-methyladenine glycosylase I
CEN28612.1	Transcription regulator
CEN28761.1	UDP-N-acetyl glucosamine-2-epimerase
CEN27783.1	DDE transposase
CEN28833.1	Putative ribosomal protein YlxQ
CEN28167.1	Hydrolase YdeN
CEN29434.1	ABC transporter permease
CEN27392.1	D-methionine-binding lipoprotein MetQ
CEN27395.1	5-formyltetrahydrofolate cyclo-ligase
CEN27260.1	Uncharacterized protein
CEN27442.1	50S ribosomal protein L33
CEN27857.1	Phosphomethylpyrimidine kinase
CEN27983.1	Branched-chain amino acid transport protein azlC
CEN28749.1	Putative Guanine/hypoxanthine permease PbuG
CEN27271.1	Type IV pilus twitching motility protein PilT
CEN28590.1	Bacteriocin
CEN29458.1	Putative HTH-type transcriptional regulator YbbH
CEN28447.1	Two-component system TCS response regulator
CEN28366.1	Two component sensor transduction histidine kinase
CEN28150.1	Uncharacterized protein
CEN27652.1	Anaerobic ribonucleoside-triphosphate reductase-activating protein
CEN27912.1	PTS system oligo-beta-mannoside-specific EIIC component
CEN29178.1	Uncharacterized protein
CEN29025.1	Predicted kinase

CEN29373.1	Putative cell surface-exposed lipoprotein [Lactococcus piscium MKFS47]
CEN28400.1	Uncharacterized protein
CEN28170.1	Uncharacterized protein
CEN29557.1	Peptide transporter
CEN28988.1	Uncharacterized protein
CEN29068.1	Putative dihydroxyacetone kinase regulator
CEN28198.1	Hyaluronan synthase
CEN27733.1	LPXTG-domain-containing protein cell wall anchor domain
CEN29057.1	Class I glutamine amidotransferase
CEN28030.1	Acyl carrier protein
CEN28009.1	MepB family protein
CEN27268.1	Putative chromosome segregation protein
CEN27935.1	CHAP domain-containing protein [Lactococcus piscium MKFS47]
CEN28813.1	Phosphoserine aminotransferase
CEN28806.1	Protein of unknown function DUF1310
CEN28256.1	DUF1934-containing protein
CEN27661.1	Polar amino acid ABC transporter inner membrane subunit
CEN29277.1	Sensor histidine kinase (Like protein to BlpH Spn)
CEN29235.1	L-rhamnose operon regulatory protein rhaS
CEN27577.1	Cell surface protein, CscA family
CEN28195.1	Transferase for other substituted phosphate groups
CEN28051.1	HTH-type transcriptional regulator DdrOP3
CEN27984.1	Branched-chain amino acid transport protein
CEN27348.1	Ribosomal-protein-alanine acetyltransferase, rimI
CEN29185.1	UDP kinase
CEN28378.1	Two-component system sensor histidine kinase
CEN29087.1	Biotin transporter BioY
CEN29014.1	Uncharacterized protein
CEN29076.1	HTH-type transcriptional regulator
CEN27281.1	Uncharacterized protein
CEN27253.1	Transport permease protein
CEN29172.1	Uncharacterized protein
CEN27582.1	Alpha-aspartyl dipeptidase Peptidase E
CEN28744.1	Amidophosphoribosyltransferase
CEN27662.1	Glutamate ABC transporter, ATP-binding protein GluA
CEN27864.1	Probable potassium transport system protein kup
CEN28959.1	Regulatory protein YeiL
CEN28719.1	Integral membrane protein
CEN27207.1	Aminoacyl-tRNA hydrolase
CEN29170.1	Uncharacterized protein
CEN27660.1	Inner membrane amino-acid ABC transporter permease protein YecS
CEN29582.1	Uncharacterized protein

CEN27982.1	Uncharacterized protein
CEN29259.1	DNA-directed RNA polymerase subunit beta
CEN27645.1	Uncharacterized protein
CEN28214.1	Transcription regulator padr n-terminal
CEN28934.1	Dephospho-CoA kinase
CEN28148.1	Uncharacterized protein
CEN27730.1	Prophage site-specific recombinase
CEN28427.1	Co-factor of ribonucleotide diphosphate reductase
CEN28906.1	ABC-type bacteriocin transporter family protein [Lactococcus piscium MKFS47]
CEN29084.1	Substrate-specific component ThiT of thiamine ECF transporter
CEN28463.1	Membrane associated protein
CEN29613.1	Uncharacterized protein
CEN28678.1	Phosphoesterase
CEN29610.1	PpGpp synthetase
CEN28779.1	Amino acid ABC transporter
CEN27801.1	Uncharacterized protein
CEN27272.1	Type IV pilin assembly protein PilC
CEN28215.1	Integral membrane protein [Lactococcus piscium MKFS47]
CEN27278.1	Acyl carrier protein (Fragment)
CEN29433.1	Putative bacteriocin export ABC transporter, lactococcin 972 group
CEN29175.1	Putative nuclease
CEN29209.1	Putative HTH-type transcriptional regulator BH0655
CEN29075.1	Transmembrane protein YjIA
CEN29035.1	Type VII secretion protein EssC
CEN28604.1	Putative metal ion ABC transporter, membrane-spanning subunit
CEN29171.1	Uncharacterized protein
CEN28618.1	Uncharacterized protein
CEN29218.1	Amidophosphoribosyltransferase
CEN28815.1	ABC transporter substrate-binding protein, amino acid
CEN28094.1	Putative ABC transporter permease component
CEN28635.1	Glyoxylase
CEN27646.1	Daunorubicin resistance ATP-binding protein drrA
CEN28158.1	HTH-type transcriptional regulator AdhR
CEN27892.1	Transcriptional regulator, TetR
CEN28172.1	Uncharacterized protein
CEN29580.1	Putative HTH-type transcriptional regulator YbFP
CEN27664.1	Uncharacterized protein
CEN28726.1	Uncharacterized protein
CEN28550.1	Methylated-DNA--protein-cysteine methyltransferase
CEN27807.1	Uncharacterized protein
CEN29361.1	AAA ATPase domain protein (Fragment)
CEN27211.1	Cell division protein DivIC

CEN29559.1	AAA ATPase domain-containing protein
CEN29579.1	Class I SAM-dependent methyltransferase
CEN28547.1	BGL operon antiterminator BglG
CEN28173.1	Cysteine methyltransferase
CEN29435.1	Putative bacteriocin [Lactococcus piscium MKFS47]
CEN28728.1	Transcriptional regulator
CEN29157.1	Phage protein YopX
CEN29432.1	Uncharacterized protein

Down regulation at 185 minutes

CEN28048.1	Heat-inducible transcriptional repressor HrcA
CEN29599.1	Acetoin dehydrogenase
CEN28936.1	Formate C-acetyltransferase
CEN27837.1	5,6-dimethylbenzimidazole synthase, bluB
CEN29594.1	Predicted oxidoreductase Myosin-crossreactive antigen ortholog
CEN29219.1	PTS fructose transporter subunit IIC
CEN28672.1	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase, dihydroxyacetone binding subunit DhaK
CEN29325.1	Universal stress protein UspA
CEN28021.1	Lipoate--protein ligase
CEN27513.1	Oxidoreductase, aldo/keto reductase
CEN28702.1	Thioredoxin
CEN28671.1	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase DhaL
CEN29223.1	Histidine-containing phosphocarrier protein of the PTS
CEN28049.1	Nucleotide exchange factor GrpE
CEN28156.1	L-threonine 3-dehydrogenase, tdh
CEN29584.1	General stress protein 24
CEN28050.1	Molecular chaperone DnaK
CEN28633.1	Negative transcriptional regulator-copper transport operon
CEN28631.1	Lead cadmium zinc and mercury transporting ATPase Copper-translocating P-type ATPase
CEN29220.1	Tagatose-6-phosphate kinase / 1-phosphofructokinase
CEN28600.1	Maltose/maltodextrin ABC superfamily ATP binding cassette transporter, binding protein
CEN28670.1	PTS system mannose-specific transporter subunit IIA
CEN27328.1	Dihydrodipicolinate reductase
CEN27424.1	Cyclopropane-fatty-acyl-phospholipid synthase
CEN29407.1	Aldehyde-alcohol dehydrogenase
CEN29275.1	Pyruvate formate-lyase-activating enzyme
CEN28022.1	Thiamine pyrophosphate-dependent dehydrogenase E1 component subunit alpha
CEN27902.1	Cellobiose-specific PTS system IIC component

CEN27794.1	Uncharacterized protein
CEN29588.1	Transglycosylase associated protein family
CEN28601.1	Maltodextrin transport system permease protein MdxF
CEN27953.1	ADP-ribose pyrophosphatase, putative
CEN27368.1	Chaperone protein ClpB
CEN27327.1	General stress protein CsbD
CEN28798.1	Uncharacterized protein
CEN28818.1	ATP-dependent endopeptidase Clp ATP-binding subunit ClpE
CEN27846.1	Threonine--tRNA ligase
CEN28303.1	Cobalamin-independent methionine synthase II
CEN27365.1	Aldo-keto reductase
CEN27656.1	Oligoendopeptidase O
CEN28661.1	Serine--tRNA ligase
CEN27906.1	Aryl-phospho-beta-D-glucosidase BglC
CEN27326.1	Molecular chaperone DnaJ
CEN27701.1	Multiple sugar-binding transport ATP-binding protein MsmK
CEN28357.1	Carboxy-cis,cis-muconate cyclase
CEN27665.1	Superoxide dismutase
CEN27886.1	Flavocytochrome c
CEN29375.1	60 kDa chaperonin
CEN29587.1	Chlorophyll synthase ChlG, chlG
CEN28023.1	PDH E1 component beta subunit
CEN28240.1	Redox-sensing transcriptional repressor Rex
CEN27538.1	NADH peroxidase Npx
CEN28092.1	Ferrous iron transport protein B
CEN28041.1	Bile salt hydrolase
CEN27980.1	Uncharacterized protein
CEN29221.1	Transcriptional regulators of sugar metabolism
CEN27655.1	Aerobic energy metabolism
CEN28063.1	ATP synthase gamma chain
CEN28025.1	Dihydrolipoyl dehydrogenase
CEN27499.1	Flavin-dependent oxidoreductase, luciferase family (Includes alkanesulfonate monooxygenase SsuD and methylene tetrahydromethanopterin reductase)
CEN27755.1	1,4-alpha-glucan branching protein GlgB
CEN28024.1	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex
CEN29586.1	Alkaline shock response membrane anchor protein AmaP
CEN27765.1	Alpha-phosphoglucomutase
CEN27758.1	Glycogen synthase
CEN27359.1	S-ribosylhomocysteine lyase
CEN28602.1	Maltose transport system permease protein malG
CEN27357.1	Phosphoglycerate kinase
CEN28699.1	Pyrimidine-nucleoside phosphorylase Pdp

CEN28239.1	Gamma-glutamyl-gamma-aminobutyrate hydrolase
CEN28075.1	Alpha-aspartyl dipeptidase Peptidase E
CEN28155.1	Universal stress protein UspA
CEN28680.1	Fructokinase/branched chain amino acid--2-keto-4-methylthiobutyrate aminotransferase
CEN29096.1	Triosephosphate isomerase
CEN28091.1	FeoA iron transporter
CEN29249.1	Aminopeptidase C (Bleomycin hydrolase)
CEN27756.1	Glucose-1-phosphate adenyltransferase
CEN28222.1	Carbamoyl-phosphate synthase large subunit
CEN29576.1	Putative iron export ATP-binding protein FetA
CEN28509.1	Alkyl hydroperoxide reductase subunit C AhpC
CEN28350.1	Glutathione peroxidase
CEN29206.1	Manganese-dependent inorganic pyrophosphatase
CEN28508.1	NADH-peroxiredoxin reductase
CEN29440.1	Uncharacterized protein
CEN28118.1	Beta-Ala-Xaa dipeptidase
CEN28349.1	Organic hydroperoxide resistance protein OhrA
CEN28598.1	Trehalose and maltose hydrolases
CEN27493.1	tRNA modification GTPase MnmE
CEN28698.1	Deoxyribose-phosphate aldolase
CEN28580.1	Predicted transcriptional regulator containing CBS domains
CEN29330.1	Aminopeptidase N
CEN28826.1	NAD-dependent DNA ligase LigA
CEN28940.1	Ribosome hibernation promoting factor
CEN29382.1	Glutamyl aminopeptidase
CEN28015.1	Oligoendopeptidase F
CEN27551.1	DNA mismatch repair protein MutS
CEN27759.1	Alpha-1,4 glucan phosphorylase
CEN28626.1	Oligo-beta-mannoside-specific phosphotransferase enzyme IIB component
CEN29053.1	C3-degrading proteinase
CEN27420.1	Lactose operon transcriptional regulator
CEN28625.1	ABC-F type ribosomal protection protein Lsa(A)
CEN27890.1	DNA mismatch repair protein MutS
CEN28064.1	ATP synthase subunit beta
CEN29222.1	Phosphoenolpyruvate-protein phosphotransferase
CEN28662.1	Phosphotransacetylase
CEN28402.1	Branched-chain-amino-acid aminotransferase
CEN27358.1	Substrate-specific component NiaX of predicted niacin ECF transporter
CEN27901.1	Phosphotransferase PTS system cellobiose lichenan-specific IIA subunit
CEN28632.1	Copper chaperone CopZ

CEN28116.1	UvrABC system protein C
CEN29208.1	Enterocin A immunity
CEN28578.1	HD superfamily metal-dependent phosphohydrolase
CEN28271.1	Uncharacterized protein
CEN28579.1	Bifunctional oligoribonuclease and PAP phosphatase NrnA
CEN28616.1	Peroxide resistance protein Dpr
CEN27422.1	Galactokinase
CEN29585.1	Bacteriocin-associated integral membrane protein
CEN28121.1	Bifunctional methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
CEN27498.1	FMN-dependent NADPH-azoreductase
CEN28046.1	D-alanyl-D-alanine metallocarboxypeptidase
CEN27490.1	ATP-dependent DNA helicase RecG
CEN29564.1	Heavy metal translocating P-type ATPase
CEN29102.1	Mannitol operon transcriptional antiterminator MtlR
CEN27558.1	DNA mismatch repair protein MutL
CEN27494.1	Beta-ketoacyl-acyl carrier protein reductase
CEN28697.1	Cytidine deaminase
CEN28363.1	Sensor protein kinase kinB
CEN27423.1	UDP-glucose--hexose-1-phosphate uridylyltransferase
CEN28062.1	ATP synthase subunit alpha
CEN27228.1	Histidinol dehydrogenase
CEN28302.1	Activator of 2-hydroxyglutaryl-CoA dehydratase
CEN27284.1	Ribosomal protein L11 methyltransferase
CEN27285.1	Ribosomal RNA small subunit methyltransferase E
CEN28045.1	Broad-specificity phosphatase
CEN28935.1	Ribose 5-phosphate isomerase
CEN28120.1	Alanine racemase
CEN27597.1	Anaerobic dehydrogenases, typically selenocysteine-containing
CEN27425.1	DNA polymerase III PolC-type
CEN28065.1	ATP synthase epsilon chain
CEN27780.1	Uracil phosphoribosyltransferase
CEN28878.1	Phosphoglycerate/bisphosphoglycerate mutase active site
CEN28040.1	NAD(FAD)-utilizing dehydrogenase, sll0175 homolog
CEN27887.1	NADPH-dependent FMN reductase
CEN28037.1	Acetyl-CoA carboxylase biotin carboxylase subunit
CEN29065.1	ABC transporter ATP-binding protein/permease
CEN27613.1	Valine--tRNA ligase
CEN27233.1	Imidazole glycerol phosphate synthase subunit HisF
CEN28468.1	N-acetylglucosamine-6-phosphate deacetylase
CEN28690.1	Adenylosuccinate lyase
CEN27502.1	Putative Transporter, major facilitator family protein
CEN27784.1	Glucose-6-phosphate 1-dehydrogenase

CEN27685.1	Thioredoxin
CEN29100.1	Mannitol-1-phosphate 5-dehydrogenase
CEN28012.1	Formate--tetrahydrofolate ligase
CEN27421.1	Aldose 1-epimerase
CEN29376.1	10 kDa chaperonin
CEN27786.1	Two-component membrane permease complex
CEN28751.1	Phage infection protein YhgE
CEN27748.1	Xaa-Pro aminopeptidase
CEN28488.1	UDP-glucose 4-epimerase GalE
CEN29561.1	CopY/TcrY family copper transport repressor (Fragment)
CEN28909.1	Mannoside-phospho-beta-d-glucosidase
CEN27757.1	Glycogen biosynthesis protein GlgD, glucose-1-phosphate adenylyltransferase family
CEN29427.1	Beta-glucoside-specific phosphotransferase enzyme IIA component
CEN28825.1	Transcription regulator [contains diacylglycerol kinase catalytic domain]
CEN28293.1	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase
CEN28270.1	Acetyltransferases, including N-acetylases of ribosomal proteins
CEN27485.1	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase
CEN28534.1	RNase adaptor protein RapZ
CEN27954.1	Ribosyl nicotinamide transporter like protein PnuC
CEN29480.1	RIP metalloprotease RseP
CEN28208.1	Transketolase
pseudo	pseudogene
CEN28060.1	ATP synthase subunit b
CEN29481.1	1-deoxy-D-xylulose 5-phosphate reductoisomerase
CEN27491.1	Short-chain dehydrogenase of various substrate specificities
CEN27567.1	DNA polymerase III subunit gamma/tau
CEN27230.1	Imidazoleglycerol-phosphate dehydratase
CEN29425.1	PTS system beta-glucoside-specific transporter subunit IIABC
CEN27641.1	Uncharacterized protein
CEN28266.1	SOS response UmuC protein
CEN28924.1	Alkaline phosphatase synthesis transcriptional regulatory protein PhoP
CEN28189.1	N-acyl-L-amino acid amidohydrolase
CEN28883.1	Thiol peroxidase
CEN27785.1	Phosphate ABC transporter substrate-binding protein
CEN27361.1	Catechol 2,3-dioxygenase
CEN29132.1	Putative phage tail protein
CEN27859.1	UvrABC system protein A
CEN27341.1	L-arabinose isomerase
CEN28359.1	Putative trans-acting positive regulator

CEN28364.1	Alkaline phosphatase synthesis transcriptional regulatory protein PhoP
CEN28800.1	Polyketide synthase regulator
CEN28939.1	Enolase
CEN28013.1	NAD-dependent oxidoreductase
CEN28220.1	Aspartate carbamoyltransferase
CEN27842.1	Divalent metal cation transporter MntH
CEN28117.1	Uncharacterized protein
CEN28715.1	Uncharacterized protein
CEN28597.1	Beta-phosphoglucomutase glucose-1-phosphate phosphodismutase
CEN28870.1	Nadh dehydrogenase
CEN29575.1	Uncharacterized protein
CEN27229.1	Phosphoserine phosphatase SerB
CEN28036.1	Beta-hydroxyacyl-ACP dehydratase
CEN29362.1	DEAD box family helicase, phage associated
CEN28221.1	Carbamoyl-phosphate synthase small subunit
CEN29103.1	Phosphotransferase system mannitol-specific IIBC component
CEN28799.1	Leucine--tRNA ligase
CEN29426.1	Aryl-phospho-beta-D-glucosidase BglH
CEN28595.1	Metal-dependent hydrolase YhfI
CEN27792.1	Cysteine sulfinase desulfurase/cysteine desulfurase
CEN27903.1	Uncharacterized protein
CEN28925.1	Cell wall metabolism sensor histidine kinase Vick
CEN28059.1	ATP synthase subunit a
CEN28035.1	Biotin carboxyl carrier protein of acetyl-CoA carboxylase
CEN29360.1	Phage replicative DNA helicase repA
CEN29231.1	Formate-nitrite transporter
CEN28811.1	Arsenate reductase ArsC
CEN29230.1	Hydrolase acting on acid anhydrides in phosphorous-containing anhydrides
CEN27232.1	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase
CEN27900.1	Carbohydrate acetyl esterase/feruloyl esterase
CEN27888.1	HTH-type transcriptional regulator CynR
CEN27234.1	Phosphoribosyl-AMP cyclohydrolase
CEN29101.1	Mannitol-specific phosphotransferase enzyme IIA component
CEN29338.1	Phage minor structural protein [Lactococcus piscium MKFS47]
CEN28753.1	DNA polymerase III alpha subunit DNA polymerase III epsilon chain
CEN28991.1	Gas vesicle protein
CEN27717.1	Helix-turn-helix domain-containing protein
CEN27707.1	Iron-sulfur cluster assembly protein SufD
CEN29257.1	Pyrroline-5-carboxylate reductase

CEN27549.1	Arginine repressor
CEN27793.1	Ribose-phosphate pyrophosphokinase 2
CEN27841.1	Metalloregulator
CEN27231.1	Imidazole glycerol phosphate synthase subunit HisH
CEN28111.1	Tryptophan synthase subunit beta
CEN28869.1	Trehalose operon transcriptional repressor
CEN27769.1	4-oxalocrotonate tautomerase Xylose transport system permease protein xylH
CEN27542.1	Glutathione-regulated potassium-efflux system ancillary protein KefG
CEN29577.1	Predicted membrane protein of the YbbM family
CEN29367.1	DNA replication and repair protein RecF
CEN28801.1	Ribose-phosphate pyrophosphokinase
CEN29569.1	Multicopper oxidase mco
CEN28157.1	HTH-type transcriptional regulator AdhR
CEN28034.1	Beta-ketoacyl-[acyl-carrier-protein] synthase II
CEN27624.1	Phage integrase site specific recombinase
CEN27342.1	Transketolase subunit A
CEN27779.1	BetaC-S lyase
CEN28216.1	4-hydroxy-tetrahydrodipicolinate synthase
CEN28466.1	Pyruvate kinase
CEN27708.1	Cysteine desulfurase
CEN27564.1	Chloride channel voltage gated
CEN28689.1	X-linked nucleoside pyrophosphate hydrolase
CEN28714.1	XRE family transcriptional regulator
CEN28817.1	Peptide deformylase
CEN29232.1	Ribose-5-phosphate isomerase A
CEN29365.1	Phage nucleotide-binding protein
CEN28309.1	Putative HTH-type transcriptional regulator YobV
CEN28069.1	Bifunctional ligase/repressor BirA
CEN27487.1	Transcriptional regulator
CEN27905.1	Na ⁺ -driven multidrug efflux pump
CEN29216.1	Phosphoglycerate dehydrogenase
CEN28642.1	Putative heme transport system permease protein HrtB
CEN28982.1	Multidomain redox protein (NAD(FAD)-dependent oxidoreductase Rhodanese domain SirA-like redox domain Peroxiredoxin domain)
CEN28016.1	Predicted O-methyltransferase YrrM
CEN27698.1	3-isopropylmalate dehydratase large subunit
CEN28109.1	Indole-3-glycerol phosphate synthase TrpC
CEN28039.1	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha
CEN29428.1	Beta-glucoside operon transcriptional antiterminator
CEN28725.1	Sucrose operon repressor ScrR

CEN28308.1	Bacterial transcription activator, effector binding domain
CEN29071.1	Predicted esterase
CEN28992.1	Methyl-accepting chemotaxis-like domain containing protein
CEN28674.1	DhaKLM operon coactivator DhaQ
CEN29210.1	Nitro/flavin reductase
CEN28329.1	Alpha/beta hydrolase
CEN28910.1	Permease IIC component
CEN29251.1	NH(3)-dependent NAD(+) synthetase
CEN29424.1	Aryl-phospho-beta-D-glucosidase BglH
CEN29252.1	Nicotinate phosphoribosyltransferase
CEN28327.1	HTH-type transcriptional activator TipA
CEN28006.1	DNA-binding protein II HBsu
CEN27226.1	ATP phosphoribosyltransferase regulatory subunit
CEN27235.1	Phosphoribosyl-ATP pyrophosphatase
CEN28313.1	Ferredoxin--NADP reductase
CEN28107.1	Anthranilate phosphoribosyltransferase
CEN28057.1	S-formylglutathione hydrolase, fghA
CEN27343.1	Transketolase subunit B
CEN29246.1	Quinone oxidoreductase 2
CEN27725.1	Coenzyme A disulfide reductase
CEN27501.1	TetR family transcriptional regulator
CEN29288.1	Protein of hypothetical function DUF1349
CEN28113.1	Tryptophan synthase subunit alpha
CEN29083.1	L-lactate dehydrogenase
CEN28441.1	Predicted 4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase
CEN28576.1	Bax inhibitor-1/YccA family protein
CEN28310.1	ClbS/DfsB family four-helix bundle protein
CEN29114.1	Prophage Lp1 protein 2
CEN27363.1	Cell surface protein, CscB family
CEN28659.1	PTS system mannose-specific IID component ManZ
CEN29078.1	MmcQ
CEN27406.1	Additional lipoprotein component of predicted cobalamin ECF transporter
CEN28416.1	Putative Msm operon regulatory protein
CEN29489.1	Plasmid partitioning protein ParB
CEN28688.1	Sucrose operon transcriptional regulator
CEN28061.1	ATP synthase subunit delta
CEN29334.1	Membrane-bound lytic murein transglycosylase D
CEN28634.1	Thiamine pyrophosphokinase
CEN27517.1	Pyridoxal phosphate-dependent aminotransferase
CEN28087.1	Peroxiredoxin
CEN28533.1	Putative gluconeogenesis factor
CEN28164.1	Phosphoglycerate mutase

CEN28658.1	PTS system mannose-specific transporter subunit IIC
CEN27950.1	Para-aminobenzoate synthase aminase component / Aminodeoxychorismate lyase
CEN28913.1	L-Cystine ABC transporter periplasmic cystine-binding protein TcyA
CEN28586.1	Riboflavin biosynthesis protein RibBA
CEN28360.1	Glycerol kinase
CEN27607.1	Transcriptional regulator YeiE
pseudo	pseudogene
CEN28298.1	Type I pantothenate kinase
CEN27407.1	Transcriptional regulator
CEN29364.1	DUF669 domain-containing protein
CEN27362.1	Cell surface protein
CEN28161.1	Predicted SAM-dependent methyltransferase
CEN27904.1	Uncharacterized protein
CEN28594.1	tRNA-specific adenosine deaminase
CEN27606.1	Putative sulfate exporter family transporter
CEN27673.1	ATP-dependent Clp protease proteolytic subunit
CEN29340.1	Phage tail length tape-measure protein
CEN27635.1	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
CEN29355.1	Transcriptional regulator
CEN27559.1	Holliday junction ATP-dependent DNA helicase RuvA
CEN28899.1	Adenosine deaminase
CEN28643.1	Membrane protein insertion efficiency factor YidD
CEN27574.1	Cell surface protein, CscB family
CEN27879.1	Xylose operon repressor
CEN28267.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN27899.1	S-formylglutathione hydrolase FrmB
CEN29289.1	Puromycin N-acetyltransferase
CEN28737.1	Glucosamine-6-phosphate deaminase
CEN28110.1	Phosphoribosylanthranilate isomerase
CEN28305.1	Nucleotidyltransferase superfamily protein
CEN27746.1	COG0596: Predicted hydrolases or acyltransferases (Alpha/beta hydrolase superfamily)
CEN29562.1	Cupredoxin domain-containing protein
CEN28465.1	Glycosyltransferase
CEN29349.1	Main capsid protein Gp34 [Lactococcus piscium MKFS47]
CEN27709.1	Nitrogen fixation protein NifU
CEN27747.1	Phosphatidylglycerol lysyltransferase
CEN27227.1	ATP phosphoribosyltransferase
CEN29072.1	Ring-cleaving dioxygenase MhqA
CEN28307.1	Bacteriocin immunity protein SkgI
CEN28504.1	Cu(I)-responsive transcriptional regulator

CEN28130.1	Putative Transcriptional regulator, Rgg/GadR/MutR family
CEN27247.1	Uncharacterized protein
CEN29144.1	Phage capsid family
CEN27514.1	IreB family regulatory phosphoprotein
CEN29565.1	Copper chaperone CopZ
CEN27895.1	Putative GTP cyclohydrolase 1 type 2, NIF3 family
CEN27344.1	Glycerol kinase
CEN28418.1	NAD binding domain of 6-phosphogluconate dehydrogenase family
CEN28536.1	N-acetylglucosamine metabolism protein NagD
CEN27724.1	Thiosulfate sulfurtransferase PspE
CEN28919.1	CBS domain-containing protein
CEN28182.1	Hydrolase MhqD
CEN27572.1	Cell surface protein, DUF208-containing
CEN28911.1	HTH-type transcriptional regulator GmuR
CEN27630.1	Aryl-phospho-beta-D-glucosidase BglA
CEN29563.1	Cupredoxin domain protein (plasmid) [Lactococcus piscium MKFS47]
CEN28918.1	Thymidylate kinase
CEN29134.1	Phage protein
CEN28496.1	Fructose-1,6-bisphosphatase class 3
CEN28984.1	Thiosulfate sulfurtransferase PspE
CEN27548.1	Phage envelope protein
CEN28886.1	Bifunctional protein: transcription regulator sugar kinase, ROK family
CEN29128.1	Bacteriophage peptidoglycan hydrolase
CEN29321.1	Predicted transcriptional regulators
CEN28502.1	Phosphopentomutase
CEN27848.1	Hydroxyacylglutathione hydrolase
CEN27353.1	Cellobiose phosphorylase
CEN29138.1	Phage major tail protein [Lactococcus piscium MKFS47]
CEN28112.1	Cytidine and deoxycytidylate deaminase zinc-binding region
CEN29346.1	Uncharacterized protein
CEN28328.1	Putative Acetyltransferase
CEN28649.1	Putative multiple-sugar transport system permease YteP
CEN27416.1	Lactose ABC superfamily ATP binding cassette transporter, binding protein
CEN29348.1	Uncharacterized protein
CEN29320.1	Uncharacterized protein
CEN28588.1	Riboflavin biosynthesis protein RibD
CEN28436.1	Diacetylchitobiose-6-phosphate hydrolase
CEN28442.1	NTP pyrophosphohydrolases containing a Zn-finger, probably nucleic-acid-binding
CEN27500.1	NADPH-dependent FMN reductase
CEN29079.1	Putative HTH-type transcriptional regulator ygzD

CEN27987.1	Phosphomethylpyrimidine kinase / Hydroxymethylpyrimidine kinase
CEN28262.1	GMP reductase
CEN28729.1	Integral membrane protein [Lactococcus piscium MKFS47]
CEN29453.1	Anhydro-N-acetylmuramic acid kinase
CEN28599.1	Neopullulanase alpha amylase subunit
CEN29405.1	HTH-type transcriptional regulator LrpB
CEN28983.1	CoA-disulfide reductase / Polysulfide binding and transferase domain
CEN27767.1	Phosphopantothenoylecysteine decarboxylase CoaC
CEN28424.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN27364.1	C-terminal membrane anchored cell surface protein
CEN27787.1	Uncharacterized protein
CEN28693.1	Phosphoribosylamine--glycine ligase
CEN29135.1	Phage minor tail protein [Lactococcus piscium MKFS47]
CEN29145.1	Phage scaffold protein
CEN27415.1	Beta-galactosidase
CEN27898.1	Transcriptional regulator AraC family
CEN29422.1	Aryl-phospho-beta-d-glucosidase
CEN29345.1	Putative phage tail component
CEN27686.1	Adenylate cyclase
CEN27496.1	Putative bacteriocin%2C class II
CEN29488.1	General stress protein GlsB
CEN27566.1	GAF domain-containing proteins
CEN29369.1	Putative phage repressor [Lactococcus piscium MKFS47]
CEN27889.1	FAD:protein FMN transferase
CEN28717.1	Uncharacterized protein
CEN27225.1	Histidinol-phosphate aminotransferase
CEN29015.1	Uncharacterized protein
CEN28730.1	Transcriptional regulator
CEN29423.1	Phosphatase YwpJ
CEN29350.1	Phage scaffold protein
CEN27544.1	Uncharacterized protein
CEN28166.1	Uncharacterized protein
CEN28774.1	Iron(III) dicitrate transport system permease protein fecD
CEN28985.1	Thiosulfate sulfurtransferase GlpE
CEN29141.1	Uncharacterized protein
CEN27339.1	LacI family transcriptional regulator
CEN28965.1	ABC transporter substrate binding component
CEN28585.1	6,7-dimethyl-8-ribityllumazine synthase
CEN28495.1	HTH-type transcriptional regulator GanR
CEN28587.1	Riboflavin synthase alpha subunit RibE
CEN29454.1	Putative carbohydrate kinase [Lactococcus piscium MKFS47]

CEN29148.1	Phage terminase large subunit
CEN27589.1	Glyoxalase
CEN27716.1	Zinc-containing alcohol dehydrogenase
CEN28904.1	M protein trans-acting positive transcriptional regulator
CEN27631.1	Phosphotransferase PTS system cellobiose lichenan-specific IIA subunit
CEN29150.1	ArpU family phage transcriptional regulator
CEN29133.1	Phage tail protein [Lactococcus piscium MKFS47]
CEN27525.1	Sorbitol operon activator protein [Lactococcus piscium MKFS47]
CEN29456.1	Permease IIC component
CEN27587.1	Enterocin A Immunity
CEN28493.1	MSM operon regulatory protein 1 MsmR1
CEN29344.1	Uncharacterized protein
CEN27593.1	Rhomboid family protein
CEN29021.1	Flavodoxin
CEN27340.1	PTS system transporter EIIB component
CEN29570.1	Transposase
CEN27952.1	Acylphosphatase
CEN27419.1	Beta-galactosidase
CEN28907.1	Putative ABC-type transporter protein [Lactococcus piscium MKFS47]
CEN27573.1	Truncated WxL domain-containing protein
CEN29366.1	Uncharacterized protein
CEN29146.1	Phage head morphogenesis protein [Lactococcus piscium MKFS47]
CEN28503.1	FMN-dependent NADH-azoreductase
CEN27634.1	Membrane-associated phospholipid phosphatase
CEN28863.1	Transcriptional regulator
CEN28596.1	Maltose operon transcriptional repressor MdxR
CEN27739.1	Anaerobic sulfatase-maturing enzyme AsIB
CEN28859.1	Predicted dehydrogenase
CEN27979.1	Peptidoglycan hydrolase
CEN29404.1	Streptothricin hydrolase
CEN27570.1	Cell surface protein%2C DUF208-containing
CEN27998.1	Phosphotransferase system cellobiose-specific component IIA

Appendix 2:

Up and down regulated genes of *L. piscium* at 28 °C

Up regulation at 5 minutes

CEN28049.1	Nucleotide exchange factor GrpE
CEN28050.1	Molecular chaperone DnaK
CEN29375.1	60 kDa chaperonin
CEN27326.1	Molecular chaperone DnaJ
CEN29376.1	10 kDa chaperonin
CEN28996.1	Uncharacterized protein YvIB
CEN27368.1	Chaperone protein ClpB
CEN28048.1	Heat-inducible transcriptional repressor HrcA
CEN29207.1	Magnesium-translocating P-type ATPase
CEN28941.1	ATP-dependent Clp protease ATP-binding subunit ClpC
CEN28818.1	ATP-dependent endopeptidase Clp ATP-binding subunit ClpE
CEN28995.1	Phage shock protein C, PspC
CEN27985.1	Bacteriocin export ABC transporter
CEN27673.1	ATP-dependent Clp protease proteolytic subunit
CEN28628.1	ABC-type multidrug transport system, ATPase and permease components
CEN28791.1	Two-component system sensor histidine kinase
CEN28469.1	Tellurite resistance protein TelA
CEN28764.1	Phosphate-specific transport system accessory protein PhoU
CEN28630.1	Putative HTH-type transcriptional regulator YdgJ
CEN28994.1	HPr kinase/phosphorylase
CEN28790.1	Two-component response regulator (YvqE) responding to cell wall stress
CEN27517.1	Pyridoxal phosphate-dependent aminotransferase
CEN28579.1	Bifunctional oligoribonuclease and PAP phosphatase NrnA
CEN28266.1	SOS response UmuC protein
CEN28792.1	Putative membrane protein YjbB [Lactococcus piscium MKFS47]
CEN28993.1	Prolipoprotein diacylglycerol transferase
CEN29219.1	PTS fructose transporter subunit IIC
CEN28345.1	Putative cell wall surface
CEN27597.1	Anaerobic dehydrogenases, typically selenocysteine-containing
CEN27725.1	Coenzyme A disulfide reductase
CEN27501.1	TetR family transcriptional regulator
CEN27327.1	General stress protein CsbD
CEN29246.1	Quinone oxidoreductase 2
CEN28942.1	Transcriptional regulator CtsR
CEN27502.1	Putative Transporter, major facilitator family protein
CEN28602.1	Maltose transport system permease protein malG
CEN29374.1	Prophage ps2 probable integrase
CEN27361.1	Catechol 2,3-dioxygenase
CEN28625.1	ABC-F type ribosomal protection protein Lsa(A)
CEN28426.1	Ribonucleoside-diphosphate reductase class Ib glutaredoxin subunit

CEN28597.1	Beta-phosphoglucomutase glucose-1-phosphate phosphodismutase
CEN27243.1	Surface antigen
CEN27572.1	Cell surface protein, DUF208-containing
CEN28361.1	Alpha-glycerophosphate oxidase
CEN27370.1	Cell surface protein%2C DUF285-containing
CEN29561.1	CopY/TcrY family copper transport repressor (Fragment)
CEN27634.1	Membrane-associated phospholipid phosphatase
CEN28360.1	Glycerol kinase
CEN28858.1	PTS system trehalose-specific transporter subunit IIBC /PTS system trehalose-specific transporter subunit IIA
CEN28770.1	Phosphate ABC transporter substrate-binding component
CEN28558.1	Integral membrane protein [Lactococcus piscium MKFS47]
CEN28346.1	Uncharacterized protein
CEN27369.1	Transcriptional regulator
CEN28271.1	Uncharacterized protein
CEN28878.1	Phosphoglycerate/bisphosphoglycerate mutase active site
CEN27247.1	Uncharacterized protein
CEN27288.1	ABC transporter ATP-binding protein YtrB

Down regulation at 5 minutes

CEN27648.1	DEAD-box ATP-dependent RNA helicase CshA
CEN27394.1	Cold shock protein CspC
CEN27519.1	Aspartate--tRNA ligase
CEN29017.1	Queuosine ECF transporter substrate-specific component QueT
CEN27295.1	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA
CEN28028.1	Transcriptional regulator of fatty acid biosynthesis FabT
CEN28191.1	Teichoic acids export ATP-binding protein TagH
CEN28086.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
CEN28608.1	Uncharacterized protein
CEN27669.1	Cysteine synthase A
CEN28850.1	Aspartokinase
CEN29309.1	DNA replication and repair protein RecF
CEN27993.1	Uncharacterized protein
CEN27661.1	Polar amino acid ABC transporter inner membrane subunit
CEN27662.1	Glutamate ABC transporter, ATP-binding protein GluA
CEN27258.1	Transcription regulator
CEN27294.1	Oligopeptide transport system permease protein AppB
CEN28285.1	Transposase
CEN29172.1	Uncharacterized protein
CEN27642.1	Aggregation promoting factor
CEN28845.1	Putative protein Veg

CEN27225.1	Histidinol-phosphate aminotransferase
CEN28043.1	ACT domain-containing protein
CEN28969.1	ATPase
CEN28876.1	Uncharacterized protein YshB
CEN28701.1	Uncharacterized protein YqjA
CEN27296.1	Glycosyltransferase involved in cell wall bisynthesis
CEN27318.1	Glycosyltransferase family protein [Lactococcus piscium MKFS47]
CEN29556.1	Replication protein RepB
CEN28387.1	CBS domain-containing protein YkuL
CEN29579.1	Class I SAM-dependent methyltransferase
CEN28782.1	L-Cystine ABC transporter periplasmic cystine-binding protein TcyA
CEN28989.1	Uncharacterized protein
CEN29609.1	Cu(I)-responsive transcriptional regulator, cueR
CEN27800.1	Negative regulator of toxin gene expression
CEN27660.1	Inner membrane amino-acid ABC transporter permease protein YecS
CEN27319.1	Glycosyl transferase
CEN27231.1	Imidazole glycerol phosphate synthase subunit HisH
CEN29234.1	Putative ABC transporter ATP-binding protein YknU
CEN29153.1	Uncharacterized protein
CEN27278.1	Acyl carrier protein (Fragment)
CEN27229.1	Phosphoserine phosphatase SerB
CEN29472.1	Uncharacterized protein
CEN29148.1	Phage terminase large subunit
CEN29163.1	DNA replication protein phage-associated
CEN28822.1	Major facilitator superfamily transporter
CEN28656.1	Hydrogen peroxide-inducible transcriptional activator
CEN27663.1	ABC transporter periplasmic protein family 3
CEN29604.1	Cadmium resistance transcriptional regulatory protein CadC
CEN27220.1	Extracellular transglycosylase
CEN29169.1	Putative DNA-binding phage protein [Lactococcus piscium MKFS47]
CEN29123.1	Phage transcriptional regulator, ArpU family
CEN27226.1	ATP phosphoribosyltransferase regulatory subunit
CEN29248.1	Multidrug resistance permease
CEN28988.1	Uncharacterized protein
CEN28742.1	DMT family transporter
CEN27857.1	Phosphomethylpyrimidine kinase
CEN29175.1	Putative nuclease
CEN29176.1	DUF739 domain-containing protein

Up regulation at 35 minutes

CEN29375.1	60 kDa chaperonin
CEN28050.1	Molecular chaperone DnaK
CEN29376.1	10 kDa chaperonin
CEN28048.1	Heat-inducible transcriptional repressor HrcA
CEN28049.1	Nucleotide exchange factor GrpE
CEN27368.1	Chaperone protein ClpB
CEN27326.1	Molecular chaperone DnaJ
CEN28818.1	ATP-dependent endopeptidase Clp ATP-binding subunit ClpE
CEN27673.1	ATP-dependent Clp protease proteolytic subunit
CEN28941.1	ATP-dependent Clp protease ATP-binding subunit ClpC
CEN27837.1	5,6-dimethylbenzimidazole synthase, bluB
CEN28022.1	Thiamine pyrophosphate-dependent dehydrogenase E1 component subunit alpha
CEN27563.1	Glutamine synthetase type III
CEN28021.1	Lipoate--protein ligase
CEN29584.1	General stress protein 24
CEN28508.1	NADH-peroxiredoxin reductase
CEN27597.1	Anaerobic dehydrogenases, typically selenocysteine-containing
CEN27665.1	Superoxide dismutase
CEN29374.1	Prophage ps2 probable integrase
CEN28942.1	Transcriptional regulator CtsR
CEN28633.1	Negative transcriptional regulator-copper transport operon
CEN28023.1	PDH E1 component beta subunit
CEN28650.1	Putative aldouronate transport system substrate-binding protein
CEN28672.1	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase, dihydroxyacetone binding subunit DhaK
CEN28424.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN29586.1	Alkaline shock response membrane anchor protein AmaP
CEN28625.1	ABC-F type ribosomal protection protein Lsa(A)
CEN27367.1	Sigma factor regulator N-terminal
CEN29588.1	Transglycosylase associated protein family
CEN28361.1	Alpha-glycerophosphate oxidase
CEN28509.1	Alkyl hydroperoxide reductase subunit C AhpC
CEN28648.1	ABC transporter permease protein LplC
CEN27502.1	Putative Transporter, major facilitator family protein
CEN27888.1	HTH-type transcriptional regulator CynR
CEN28619.1	DNA polymerase IV
CEN28628.1	ABC-type multidrug transport system, ATPase and permease components
CEN29585.1	Bacteriocin-associated integral membrane protein
CEN27247.1	Uncharacterized protein
CEN28649.1	Putative multiple-sugar transport system permease YteP
CEN28671.1	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase DhaL

CEN29572.1	Site-specific DNA recombinase
CEN29126.1	Uncharacterized protein
CEN28670.1	PTS system mannose-specific transporter subunit IIA
CEN28720.1	Putative quinolone resistance protein [Lactococcus piscium MKFS47]
CEN28630.1	Putative HTH-type transcriptional regulator YdgJ
CEN28360.1	Glycerol kinase
CEN28983.1	CoA-disulfide reductase / Polysulfide binding and transferase domain
CEN28007.1	N-acetylmuramoyl-L-alanine amidase
CEN27717.1	Helix-turn-helix domain-containing protein
CEN29618.1	Uncharacterized protein
CEN28559.1	Regulatory protein MarR
CEN29220.1	Tagatose-6-phosphate kinase / 1-phosphofructokinase
CEN27501.1	TetR family transcriptional regulator
CEN27590.1	Predicted membrane protein
CEN28329.1	Alpha/beta hydrolase
CEN28959.1	Regulatory protein YeiL
CEN28427.1	Co-factor of ribonucleotide diphosphate reductase
CEN28647.1	DUF624-containing protein
CEN27981.1	GNAT family N-acetyltransferase
CEN28675.1	Putative MarR family transcription regulator [Lactococcus piscium MKFS47]
CEN28493.1	MSM operon regulatory protein 1 MsmR1
CEN27527.1	PTS system sorbitol-specific transporter IIBC component SrlE
CEN29615.1	Group II intron-interrupted relaxase LtrB
CEN28362.1	Channel protein, MIP family
CEN27790.1	Transposase
CEN28346.1	Uncharacterized protein
CEN28052.1	Uncharacterized protein
CEN27438.1	Predicted membrane protein
CEN27569.1	Cell surface protein%2C DUF208-containing
CEN27372.1	Uncharacterized protein
CEN27630.1	Aryl-phospho-beta-D-glucosidase BglA
CEN27618.1	Uncharacterized protein
CEN28708.1	Phage transcriptional activator
CEN28083.1	Anticodon nuclease PrrC
CEN28328.1	Putative Acetyltransferase
CEN28494.1	Alpha-galactosidase
pseudo	pseudogene

Down regulation at 35 minutes

CEN27648.1	DEAD-box ATP-dependent RNA helicase CshA
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CEN28114.1	Ribosomal protein S1 RpsA
CEN27394.1	Cold shock protein CspC
CEN29017.1	Queuosine ECF transporter substrate-specific component QueT
CEN28029.1	Ketoacyl-ACP synthase III
CEN28028.1	Transcriptional regulator of fatty acid biosynthesis FabT
CEN28679.1	DUF402 domain-containing protein
CEN29016.1	Glutathione peroxidase
CEN28086.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG
CEN27669.1	Cysteine synthase A
CEN29233.1	ABC transporter, ATPase activity
CEN28874.1	Nanthine-uracil permease
CEN29148.1	Phage terminase large subunit
CEN29134.1	Phage protein
CEN29114.1	Prophage Lp1 protein 2
CEN29234.1	Putative ABC transporter ATP-binding protein YknU
CEN29145.1	Phage scaffold protein
CEN28749.1	Putative Guanine/hypoxanthine permease PbuG
CEN27693.1	Arginine ABC transporter ATP-binding protein ArtP
CEN28750.1	Predicted hydrolases of the HAD superfamily
CEN29147.1	Portal protein, phage associated
CEN27292.1	Oligopeptide/dipeptide uptake family ABC transporter, ATP-binding protein
CEN27291.1	Oligopeptide ABC transporter ATP-binding protein OppF
CEN29132.1	Putative phage tail protein
CEN27295.1	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA
CEN29144.1	Phage capsid family
CEN29135.1	Phage minor tail protein [Lactococcus piscium MKFS47]
CEN28875.1	Xanthine phosphoribosyltransferase
CEN27348.1	Ribosomal-protein-alanine acetyltransferase, rimI
CEN27660.1	Inner membrane amino-acid ABC transporter permease protein YecS
CEN27405.1	Succinyl-CoA synthetase alpha subunit-related enzyme
CEN28603.1	Uncharacterized protein
CEN28850.1	Aspartokinase
CEN29146.1	Phage head morphogenesis protein [Lactococcus piscium MKFS47]
CEN27294.1	Oligopeptide transport system permease protein AppB
CEN29138.1	Phage major tail protein [Lactococcus piscium MKFS47]
CEN29139.1	Major structural protein 2
CEN29137.1	Phage protein
CEN27662.1	Glutamate ABC transporter, ATP-binding protein GluA
CEN27293.1	Oligopeptide transport system permease protein AppC or OppC
CEN27663.1	ABC transporter periplasmic protein family 3

CEN28822.1	Major facilitator superfamily transporter
CEN29141.1	Uncharacterized protein
CEN29133.1	Phage tail protein [Lactococcus piscium MKFS47]

Up regulation at 185 minutes

CEN28818.1	ATP-dependent endopeptidase Clp ATP-binding subunit ClpE
CEN27368.1	Chaperone protein ClpB
CEN29619.1	SOS response UmuC protein
CEN27618.1	Uncharacterized protein
CEN28424.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN29375.1	60 kDa chaperonin
CEN28650.1	Putative aldouronate transport system substrate-binding protein
CEN29584.1	General stress protein 24
CEN27499.1	Flavin-dependent oxidoreductase, luciferase family (Includes alkanesulfonate monooxygenase SsuD and methylene tetrahydromethanopterin reductase)
CEN29585.1	Bacteriocin-associated integral membrane protein
CEN28050.1	Molecular chaperone DnaK
CEN28631.1	Lead cadmium zinc and mercury transporting ATPase Copper-translocating P-type ATPase
CEN28423.1	Glutamine--fructose-6-phosphate aminotransferase
CEN29586.1	Alkaline shock response membrane anchor protein AmaP
CEN28633.1	Negative transcriptional regulator-copper transport operon
CEN27500.1	NADPH-dependent FMN reductase
CEN28996.1	Uncharacterized protein YvIB
CEN28751.1	Phage infection protein YhgE
CEN29376.1	10 kDa chaperonin
CEN29562.1	Cupredoxin domain-containing protein
CEN29561.1	CopY/TcrY family copper transport repressor (Fragment)
CEN28355.1	Zinc ABC transporter substrate-binding protein AdcA
CEN28648.1	ABC transporter permease protein LplC
CEN27837.1	5,6-dimethylbenzimidazole synthase, bluB
CEN28913.1	L-Cystine ABC transporter periplasmic cystine-binding protein TcyA
CEN27954.1	Ribosyl nicotinamide transporter like protein PnuC
CEN27585.1	Uncharacterized protein
CEN27953.1	ADP-ribose pyrophosphatase, putative
CEN29564.1	Heavy metal translocating P-type ATPase
CEN29618.1	Uncharacterized protein
CEN28649.1	Putative multiple-sugar transport system permease YteP
CEN28675.1	Putative MarR family transcription regulator [Lactococcus piscium MKFS47]

CEN29563.1	Cupredoxin domain protein (plasmid) [Lactococcus piscium MKFS47]
CEN28601.1	Maltodextrin transport system permease protein MdxF
CEN27981.1	GNAT family N-acetyltransferase
CEN28708.1	Phage transcriptional activator
CEN27712.1	ATPase component BioM of energizing module of biotin ECF transporter
CEN28718.1	Tyrosine recombinase XerD
CEN27498.1	FMN-dependent NADPH-azoreductase
CEN29072.1	Ring-cleaving dioxygenase MhqA
CEN28092.1	Ferrous iron transport protein B
CEN28049.1	Nucleotide exchange factor GrpE
CEN29053.1	C3-degrading proteinase
CEN29220.1	Tagatose-6-phosphate kinase / 1-phosphofructokinase
CEN28361.1	Alpha-glycerophosphate oxidase
CEN28004.1	Spore germination lipase LipC
CEN27839.1	Glycine betaine ABC transport system ATP-binding protein OpuAA
CEN28639.1	ATP-dependent DNA helicase RecQ
CEN28646.1	Dextran glucosidase
CEN27840.1	Glycine betaine ABC transporter permease and substrate-binding component
CEN28720.1	Putative quinolone resistance protein [Lactococcus piscium MKFS47]
CEN28715.1	Uncharacterized protein
CEN28967.1	Two-component sensor histidine kinase YesM
CEN28714.1	XRE family transcriptional regulator
CEN28262.1	GMP reductase
CEN28820.1	DNA primase
CEN28959.1	Regulatory protein YeiL
CEN29078.1	MmcQ
CEN28647.1	DUF624-containing protein
CEN29077.1	Fhu operon transcriptional regulator
CEN28969.1	ATPase
CEN27420.1	Lactose operon transcriptional regulator
CEN27595.1	Single-stranded DNA-binding protein 2
CEN28995.1	Phage shock protein C, PspC
CEN28619.1	DNA polymerase IV
CEN29374.1	Prophage ps2 probable integrase
CEN28481.1	Poly(glycerophosphate chain) D-alanine transfer protein DltD
CEN27673.1	ATP-dependent Clp protease proteolytic subunit
CEN29073.1	Putative flavoprotein oxygenase
CEN28632.1	Copper chaperone CopZ
CEN28328.1	Putative Acetyltransferase
CEN28359.1	Putative trans-acting positive regulator

CEN27339.1	LacI family transcriptional regulator
CEN27438.1	Predicted membrane protein
CEN28625.1	ABC-F type ribosomal protection protein Lsa(A)
CEN28360.1	Glycerol kinase
CEN28397.1	Site-specific tyrosine recombinase
CEN29071.1	Predicted esterase
CEN27341.1	L-arabinose isomerase
CEN27714.1	Lactoylglutathione lyase
CEN29052.1	Beta-lactamase class C and other penicillin binding proteins
CEN28673.1	Dihydroxyacetone kinase transcriptional activator DhaS
CEN27776.1	Regulatory protein, TetR
CEN27342.1	Transketolase subunit A
CEN29615.1	Group II intron-interrupted relaxase LtrB
CEN27963.1	Uncharacterized protein
CEN28439.1	30S ribosomal protein S9
CEN29126.1	Uncharacterized protein
CEN27717.1	Helix-turn-helix domain-containing protein
CEN29617.1	Uncharacterized protein
CEN28438.1	Glutathionyl-hydroquinone reductase YqjG
CEN29356.1	Uncharacterized protein
CEN28717.1	Uncharacterized protein
CEN28379.1	Sigma-70 region 2
CEN28200.1	UDP-N-acetylenolpyruvoylglucosamine reductase
CEN27321.1	Nucleotide sugar dehydrogenase
CEN27343.1	Transketolase subunit B
CEN28166.1	Uncharacterized protein
CEN28716.1	Uncharacterized protein
CEN29217.1	HTH-type transcriptional regulator IrpC
CEN28539.1	Oxygen-independent coproporphyrinogen-III oxidase-like protein
CEN27586.1	Malolactic fermentation system transcriptional regulator MleR
CEN27777.1	LacX protein plasmid
CEN27214.1	Beta-lactamase-type transpeptidase YacG
CEN28655.1	Pore forming protein ebsA
CEN28329.1	Alpha/beta hydrolase
CEN27222.1	Membrane-associated phospholipid phosphatase
CEN28327.1	HTH-type transcriptional activator TipA
CEN29612.1	Mercuric resistance operon regulatory protein MerR
CEN28263.1	30S ribosomal protein S16
CEN29556.1	Replication protein RepB
CEN28251.1	TetR/AcrR family transcriptional regulator
CEN29613.1	Uncharacterized protein
CEN27215.1	tRNA(Ile)-lysidine synthase
CEN27543.1	TetR family HTH transcriptional regulator

CEN29363.1	Uncharacterized protein
CEN27931.1	Putative HTH-type transcriptional repressor AseR
CEN28667.1	4Fe-4S ferredoxin
CEN27932.1	Predicted permease duf318
CEN28966.1	Two-component response regulator YesN
CEN27344.1	Glycerol kinase
CEN29566.1	Prolipoprotein diacylglyceryl transferase
CEN29079.1	Putative HTH-type transcriptional regulator ygzD
CEN28238.1	Integral membrane protein [Lactococcus piscium MKFS47]
CEN27803.1	Uncharacterized protein
CEN29246.1	Quinone oxidoreductase 2
CEN29002.1	Pyridoxal phosphate homeostasis protein
CEN27210.1	RNA-binding S4 domain-containing protein
CEN28005.1	YfaA
CEN29422.1	Aryl-phospho-beta-d-glucosidase
CEN28983.1	CoA-disulfide reductase / Polysulfide binding and transferase domain
CEN28612.1	Transcription regulator
CEN28989.1	Uncharacterized protein
CEN28083.1	Anticodon nuclease PrrC
CEN27406.1	Additional lipoprotein component of predicted cobalamin ECF transporter
CEN29319.1	NifS/lcsS protein homolog
CEN29015.1	Uncharacterized protein
CEN28906.1	ABC-type bacteriocin transporter family protein [Lactococcus piscium MKFS47]
CEN27340.1	PTS system transporter EIIB component
CEN28730.1	Transcriptional regulator
CEN29368.1	Uncharacterized protein
CEN28582.1	Protein of unknown function DUF1361
CEN28755.1	Xaa-Pro aminopeptidase
CEN27867.1	Malolactic fermentation system transcriptional regulator MleR
CEN28054.1	Sodium ABC transporter permease
CEN28264.1	KH domain-containing protein
CEN28252.1	Sugar phosphate phosphatase
CEN28630.1	Putative HTH-type transcriptional regulator YdgJ
CEN27630.1	Aryl-phospho-beta-D-glucosidase BglA
CEN27620.1	Uncharacterized protein
CEN27993.1	Uncharacterized protein
CEN29616.1	Bacterial mobilization protein (MobC) family protein PcfF
CEN28210.1	Negative transcription regulator PadR
CEN27572.1	Cell surface protein, DUF208-containing pseudo
	pseudogene
CEN29405.1	HTH-type transcriptional regulator LrpB

CEN28615.1	DUF910 domain-containing protein
CEN27845.1	Manganese/zinc ABC transporter, substrate binding protein
CEN28198.1	Hyaluronan synthase
CEN29291.1	Cyclic nucleotide-binding domain-containing protein
CEN28624.1	Threonine/homoserine efflux transporter RhtA
CEN28828.1	Beta-glucoside operon transcriptional antiterminator
CEN29180.1	Uncharacterized protein
CEN28701.1	Uncharacterized protein YqjA
CEN28681.1	PTS system sucrose-specific transporter subunits IIBC A
CEN28987.1	Uncharacterized protein
CEN29213.1	Transcriptional regulators
CEN27253.1	Transport permease protein
CEN29609.1	Cu(I)-responsive transcriptional regulator, cueR
CEN27781.1	Uncharacterized protein
CEN28007.1	N-acetylmuramoyl-L-alanine amidase
CEN28051.1	HTH-type transcriptional regulator DdrOP3
CEN27744.1	Brp/Blh family beta-carotene 15,15'-monooxygenase
CEN28380.1	Uncharacterized protein
CEN28426.1	Ribonucleoside-diphosphate reductase class Ib glutaredoxin subunit
CEN28142.1	Cell division protein FtsL
CEN27322.1	Exopolysaccharide biosynthesis protein EpsL
CEN29170.1	Uncharacterized protein
CEN28070.1	PRD/PTS system IIA 2 domain protein
CEN28425.1	Glycerol-3-phosphate acyltransferase
CEN27799.1	Prophage site-specific recombinase
CEN28130.1	Putative Transcriptional regulator, Rgg/GadR/MutR family
CEN28047.1	Uncharacterized protein YjhH
CEN27958.1	Uncharacterized protein
CEN29314.1	Regulatory protein, luxR family
CEN29295.1	Formamidopyrimidine-DNA glycosylase
CEN28202.1	Uncharacterized protein
CEN29165.1	Phage-associated recombinase
CEN27596.1	30S ribosomal protein S18
CEN28233.1	Iron compound ABC uptake transporter substrate-binding protein
CEN28273.1	HTH-type transcriptional regulator ImmR
CEN29580.1	Putative HTH-type transcriptional regulator YbfP
CEN28052.1	Uncharacterized protein
CEN28860.1	Inosose isomerase
CEN28618.1	Uncharacterized protein
CEN28910.1	Permease IIC component
CEN28362.1	Channel protein, MIP family
CEN28493.1	MSM operon regulatory protein 1 MsmR1

CEN28583.1	Uncharacterized protein
CEN29438.1	Ribosomal RNA large subunit methyltransferase H
CEN27715.1	Hydroxyacylglutathione hydrolase
CEN27959.1	Pleiotropic regulator of exopolysaccharide synthesis, competence and biofilm formation Ftr, XRE family
CEN28186.1	Uncharacterized protein
CEN27733.1	LPXTG-domain-containing protein cell wall anchor domain
CEN28477.1	Teichoic acid D-Ala incorporation-associated protein DltX
CEN28193.1	Transferase for other substituted phosphate groups
CEN28427.1	Co-factor of ribonucleotide diphosphate reductase
CEN27320.1	Polysaccharide biosynthesis export protein [Lactococcus piscium MKFS47]
CEN28856.1	Trypsin_2/MucBP domain-containing protein
CEN28973.1	Class I SAM-dependent methyltransferase
CEN28922.1	Glutamine transport glutamine-binding protein GlnH
CEN27369.1	Transcriptional regulator
CEN27621.1	FtsK/SpoIIIE family protein
CEN28387.1	CBS domain-containing protein YkuL
CEN29175.1	Putative nuclease
CEN28345.1	Putative cell wall surface
CEN29118.1	ABC-type dipeptide transport system, periplasmic component
CEN29462.1	Cysteine ABC transporter, substrate-binding protein
CEN28153.1	HTH-type transcriptional regulator YofA
CEN28009.1	MepB family protein
CEN28756.1	HTH-type transcriptional repressor GlcR
CEN28285.1	Transposase
CEN28845.1	Putative protein Veg
CEN28358.1	AI-2E family transporter
CEN28494.1	Alpha-galactosidase
CEN29392.1	Glycosyltransferase RgpE
CEN29373.1	Putative cell surface-exposed lipoprotein [Lactococcus piscium MKFS47]
CEN28078.1	Oligopeptide ABC transporter periplasmic oligopeptide-binding protein OppA
CEN28196.1	Glycosyl transferase, group 2 family protein
CEN29173.1	Antirepressor
CEN28431.1	ABC-type transport system, multidrug-family ATP-binding/permease protein
CEN29572.1	Site-specific DNA recombinase
CEN29372.1	Uncharacterized protein
CEN29163.1	DNA replication protein phage-associated
CEN28691.1	5-(Carboxyamino)imidazole ribonucleotide synthase
CEN28754.1	N-acetyltransferase
CEN28430.1	Putative ABC transporter, ATP-binding/permease protein
CEN28376.1	Bacitracin export permease protein BceB

CEN28000.1	Cellobiose-specific phosphotransferase system enzyme IIB component
CEN29332.1	NrdH-redoxin
CEN28392.1	Riboflavin transporter RibU
CEN28480.1	D-alanyl carrier protein
CEN27741.1	UPF0398 protein
CEN28862.1	Predicted dehydrogenase
CEN27995.1	PTS cellobiose transporter subunit IIC

Down regulation at 185 minutes

EN29407.1	Aldehyde-alcohol dehydrogenase
CEN27846.1	Threonine--tRNA ligase
CEN28064.1	ATP synthase subunit beta
CEN28658.1	PTS system mannose-specific transporter subunit IIC
CEN28065.1	ATP synthase epsilon chain
CEN29222.1	Phosphoenolpyruvate-protein phosphotransferase
CEN27860.1	Pyruvate-flavodoxin oxidoreductase
CEN28062.1	ATP synthase subunit alpha
CEN29223.1	Histidine-containing phosphocarrier protein of the PTS
CEN28657.1	Mannose-specific phosphotransferase system component iiab
CEN29012.1	S-adenosylmethionine synthase
CEN27886.1	Flavocytochrome c
CEN29114.1	Prophage Lp1 protein 2
CEN29480.1	RIP metalloprotease RseP
CEN29253.1	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
CEN29074.1	Phosphoglycerate mutase
CEN28303.1	Cobalamin-independent methionine synthase II
CEN27792.1	Cysteine sulfinase/cysteine desulfurase
CEN29215.1	Lysine--tRNA ligase
CEN28569.1	Phosphonate ABC transporter ATPase
CEN28680.1	Fructokinase/branched chain amino acid--2-keto-4-methylthiobutyrate aminotransferase
CEN27793.1	Ribose-phosphate pyrophosphokinase 2
CEN28012.1	Formate--tetrahydrofolate ligase
CEN28241.1	ATPase component of ABC transporter with duplicated ATPase domains
CEN28243.1	Dihydroorotase
CEN28877.1	50S ribosomal protein L31 type B
CEN29251.1	NH(3)-dependent NAD(+) synthetase
CEN28566.1	Elongation factor 4
CEN29100.1	Mannitol-1-phosphate 5-dehydrogenase
CEN28468.1	N-acetylglucosamine-6-phosphate deacetylase
CEN28546.1	DNA gyrase subunit A

CEN29066.1	ABC transporter ATP-binding protein/permease
CEN27521.1	Glutamyl-tRNA(Gln) amidotransferase subunit A
CEN29382.1	Glutamyl aminopeptidase
CEN27519.1	Aspartate--tRNA ligase
CEN28961.1	Ribosome biogenesis GTPase YqeH
CEN28567.1	Nucleoside pyrophosphate kinase
CEN29272.1	Protein phosphatase PhpP
CEN28013.1	NAD-dependent oxidoreductase
CEN29331.1	Adenylosuccinate synthetase
CEN27478.1	Dihydroxy-acid dehydratase
CEN27491.1	Short-chain dehydrogenase of various substrate specificities
CEN27950.1	Para-aminobenzoate synthase aminase component / Aminodeoxychorismate lyase
CEN28066.1	L-serine dehydratase, iron-sulfur-dependent subunit alpha
CEN29134.1	Phage protein
CEN29234.1	Putative ABC transporter ATP-binding protein YknU
CEN28250.1	UPF0135 protein Bsu YqfO
CEN29016.1	Glutathione peroxidase
CEN29132.1	Putative phage tail protein
CEN27767.1	Phosphopantothenoylcysteine decarboxylase CoaC
CEN29255.1	ADP-ribose pyrophosphatase
CEN29008.1	Primosomal protein N'
CEN27261.1	Pyridoxal phosphate-dependent aminotransferase
CEN28800.1	Polyketide synthase regulator
CEN29103.1	Phosphotransferase system mannitol-specific IIBC component
CEN29274.1	Methionyl-tRNA formyltransferase
CEN27332.1	Nicotinate-nicotinamide nucleotide adenylyltransferase
CEN29323.1	Sodium:hydrogen antiporter
CEN29101.1	Mannitol-specific phosphotransferase enzyme IIA component
CEN27361.1	Catechol 2,3-dioxygenase
CEN27743.1	Penicillin-binding protein 1A
CEN29017.1	Queuosine ECF transporter substrate-specific component QueT
CEN27698.1	3-isopropylmalate dehydratase large subunit
CEN27402.1	Probable dual-specificity RNA methyltransferase RlmN
CEN29138.1	Phage major tail protein [Lactococcus piscium MKFS47]
CEN28570.1	Branched-chain amino acid ABC transporter permease
CEN29208.1	Enterocin A immunity
CEN28873.1	Phenylalanine--tRNA ligase alpha subunit
CEN27887.1	NADPH-dependent FMN reductase
CEN27602.1	23S rRNA (Uracil-5-)-methyltransferase RumA
CEN27579.1	High affinity arginine ABC transporter (ATP-binding protein)
CEN29256.1	Bifunctional UDP-N-acetylglucosamine diphosphorylase/glucosamine-1-phosphate N-acetyltransferase GlmU

CEN27393.1	Damage-inducible protein DinF
CEN28734.1	Xaa-Pro dipeptidase
CEN28523.1	Trk system potassium uptake protein TrkH
CEN27264.1	Metal-dependent phosphohydrolase
CEN28947.1	Single-stranded-DNA-specific exonuclease RecJ
CEN27405.1	Succinyl-CoA synthetase alpha subunit-related enzyme
CEN28441.1	Predicted 4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase
CEN29416.1	D-alanyl-D-alanine serine-type carboxypeptidase
CEN29135.1	Phage minor tail protein [Lactococcus piscium MKFS47]
CEN27632.1	Putative nodulation ATP-binding protein I
CEN27855.1	Reactive intermediate/imine deaminase
CEN28011.1	Sugar efflux transporter for intercellular exchange
CEN27610.1	YbaB/EbfC family nucleoid-associated protein
CEN27669.1	Cysteine synthase A
CEN27842.1	Divalent metal cation transporter MntH
CEN28812.1	Phosphoglycerate dehydrogenase
CEN29047.1	Class I SAM-dependent methyltransferase
CEN29095.1	Uncharacterized protein YuhC
CEN28247.1	DNA replication protein DnaD
CEN27769.1	4-oxalocrotonate tautomerase Xylose transport system permease protein xylH
CEN29146.1	Phage head morphogenesis protein [Lactococcus piscium MKFS47]
CEN29148.1	Phage terminase large subunit
CEN29133.1	Phage tail protein [Lactococcus piscium MKFS47]
CEN29021.1	Flavodoxin
CEN27263.1	Glycerophosphodiester phosphodiesterase
CEN28568.1	Uncharacterized protein YngA
CEN28962.1	YqeG family HAD IIIA-type phosphatase
CEN29009.1	DNA-directed RNA polymerase subunit omega
CEN27702.1	Ectoine/hydroxyectoine ABC transporter, permease protein EhuC, ehuC
CEN29010.1	Guanylate kinase
CEN29233.1	ABC transporter, ATPase activity
CEN28068.1	Phosphoglycolate phosphatase
CEN27235.1	Phosphoribosyl-ATP pyrophosphatase
CEN27705.1	Undecaprenyl-phosphate alpha-N-acetylglucosaminephosphotransferase
CEN27696.1	3-isopropylmalate dehydrogenase
CEN28232.1	Phosphoribosyl formyl glycinamidine synthase
CEN29144.1	Phage capsid family
CEN27841.1	Metallorepressor
CEN29344.1	Uncharacterized protein
CEN28473.1	Protein export element

CEN29145.1	Phage scaffold protein
CEN28020.1	Putative AB hydrolase superfamily protein YdjP
CEN27512.1	50S ribosomal protein L34
CEN28299.1	Esterase
CEN27267.1	Predicted hydrolases of the HAD superfamily
CEN27308.1	Acyl-phosphate glycerol 3-phosphate acyltransferase
CEN28297.1	3-dehydroquinase dehydratase I
CEN27601.1	PpGpp synthetase
CEN27770.1	Small ribosomal subunit biogenesis GTPase RsgA
CEN27304.1	High-affinity branched-chain amino acid ABC transporter permease LivM
CEN27609.1	Putative aminoacid permease
CEN28497.1	Ribokinase
CEN27415.1	Beta-galactosidase
CEN28517.1	Pseudouridine synthase
CEN29128.1	Bacteriophage peptidoglycan hydrolase
CEN28296.1	LSU methyltransferase RlmI
CEN27861.1	Diadenylate cyclase
CEN29018.1	Conserved membrane protein, GtcA family
CEN28442.1	NTP pyrophosphohydrolases containing a Zn-finger, probably nucleic-acid-binding
CEN27608.1	Homocysteine S-methyltransferase
CEN27298.1	D-aminoacyl-tRNA deacylase
CEN27603.1	N-acetyltransferase
CEN27480.1	Acetolactate synthase catalytic subunit
CEN28182.1	Hydrolase MhqD
CEN28515.1	Two-component system KDP operon response regulator KdpE
CEN28086.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG

Appendix 3:

Pathway enrichment and metabolism changes of *L. piscium*

0 – 35 (upregulated)

Name	P-value
Adenine and adenosine salvage	5.16e-03
Purine nucleotide salvage	5.16e-03

L-alanine degradation	2.41e-02
L-alanine degradation iv	2.41e-02
Udp-α-d-glucuronate biosynthesis (from udp-glucose)	2.41e-02
Adenine and adenosine salvage i	4.77e-02
Aldehyde degradation	4.77e-02
Chitin degradation	4.77e-02
Chitin degradation ii (vibrio)	4.77e-02
Glycerol-3-phosphate to cytochrome <i>bo</i> oxidase electron transfer	4.77e-02
Methylglyoxal degradation i	4.77e-02
Methylglyoxal detoxification	4.77e-02
Polymeric compounds degradation	4.77e-02
Polysaccharides degradation	4.77e-02
Xanthine and xanthosine salvage	4.77e-02

0 – 35 (downregulated)

Name	Data
Ump biosynthesis	3.45e-05
Nucleosides and nucleotides biosynthesis	7.90e-05
Pyrimidine ribonucleotides <i>de novo</i> biosynthesis	4.53e-04
2,3-butanediol degradation	2.80e-03
Fermentation	4.83e-03
2'-deoxyribonucleotides biosynthesis	5.39e-03
Pyrimidine nucleotides <i>de novo</i> biosynthesis	5.39e-03
Fermentation to alcohols	6.55e-03
Sugars biosynthesis	6.55e-03

Fermentation of pyruvate	9.39e-03
Carbohydrates biosynthesis	1.30e-02
Glycolysis iii (from glucose)	1.30e-02
Superpathway of purine nucleotides <i>de novo</i> biosynthesis i	1.51e-02
L-cysteine biosynthesis vi (from L-methionine)	1.57e-02
Generation of precursor metabolites and energy	1.70e-02
Adenosine ribonucleotides <i>de novo</i> biosynthesis	1.73e-02
Gluconeogenesis	1.73e-02
Homolactic fermentation	1.73e-02
L-methionine biosynthesis	1.73e-02
Purine nucleotides <i>de novo</i> biosynthesis	2.00e-02
Purine ribonucleotides <i>de novo</i> biosynthesis	2.14e-02
Fermentation to lactate	2.52e-02
<i>s</i> -adenosyl-L-methionine cycle i	2.53e-02
L-methionine biosynthesis i	2.53e-02
Pyruvate fermentation to (<i>r</i>)-acetoin i	2.53e-02
Pyruvate fermentation to (<i>s</i>)-acetoin	2.53e-02
Sugar nucleotides biosynthesis	3.42e-02
Purine nucleotide biosynthesis	3.66e-02
Butanediol biosynthesis	3.67e-02
L-cysteine biosynthesis	3.67e-02
Pyruvate fermentation to acetoin	3.67e-02
Superpathway of (<i>r,r</i>)-butanediol biosynthesis	3.67e-02
Urea cycle	3.67e-02
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	4.12e-02
Fermentation to short-chain fatty acids	4.42e-02
Heterolactic fermentation	4.89e-02

Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis ii	4.89e-02
D-galactose degradation i (leloir pathway)	4.96e-02
D-galactose degradation v (leloir pathway)	4.96e-02
Galactose degradation	4.96e-02

0 – 185 (upregulated)

Name	P-value
Poly(glycerol phosphate) wall teichoic acid biosynthesis	7.76e-06
Teichoic acids biosynthesis	7.76e-06
Cell structures biosynthesis	3.15e-05
Cell wall biosynthesis	3.15e-05
Purine nucleotide biosynthesis	2.49e-03
Nucleosides and nucleotides biosynthesis	5.21e-03
5-aminoimidazole ribonucleotide biosynthesis	1.01e-02
Antibiotic resistance	1.29e-02
Peptidoglycan biosynthesis v (β -lactam resistance)	1.29e-02
Superpathway of purine nucleotides <i>de novo</i> biosynthesis i	1.52e-02
Peptidoglycan biosynthesis	1.83e-02
Heptaprenyl diphosphate biosynthesis	2.07e-02
Trna processing	2.07e-02
Adenine and adenosine salvage	2.35e-02
Glycerophosphodiester degradation	2.35e-02
Purine nucleotide salvage	2.35e-02
Utp and ctp <i>de novo</i> biosynthesis	2.35e-02
Detoxification	2.80e-02

0 – 185 (downregulated)

Name	P-value
L-histidine biosynthesis	1.99e-05
Sugars degradation	5.85e-04
Carbohydrates degradation	1.37e-03
Adenosine ribonucleotides <i>de novo</i> biosynthesis	2.41e-03
Chitobiose degradation	2.41e-03
Superpathway of pyrimidine deoxyribonucleosides degradation	2.41e-03
Pyruvate fermentation to ethanol	2.90e-03
Heterolactic fermentation	3.64e-03
Fermentation of pyruvate	3.95e-03
Fermentation to alcohols	5.17e-03
Fermentation to lactate	6.75e-03
Generation of precursor metabolites and energy	7.13e-03
Vitamins biosynthesis	7.16e-03
5,6-dimethylbenzimidazole biosynthesis	7.62e-03
5,6-dimethylbenzimidazole biosynthesis i (aerobic)	7.62e-03
Cobalamin biosynthesis	7.62e-03
Fermentation to short-chain fatty acids	1.24e-02
Fermentation	1.49e-02
D-galactose degradation i (leloir pathway)	2.06e-02
D-galactose degradation v (leloir pathway)	2.06e-02
Galactose degradation	2.06e-02
L-tryptophan biosynthesis	2.06e-02
L-tryptophan biosynthesis	2.06e-02
<i>n</i> -acetylglucosamine degradation	2.17e-02

Pyrimidine deoxyribonucleosides degradation	2.17e-02
Acetyl-coa biosynthesis	2.33e-02
Glycogen and starch biosynthesis	2.33e-02
Glycogen biosynthesis i (from adp-d-glucose)	2.33e-02
Polysaccharides biosynthesis	2.33e-02
Pyruvate decarboxylation to acetyl coa	2.33e-02
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	3.19e-02

4 – 35 (upregulated)

Name	P-val
Amines and polyamines biosynthesis	2.44e-03
Ectoine biosynthesis	2.44e-03
L-homoserine biosynthesis	2.44e-03
Purine nucleotide biosynthesis	2.56e-03
Adenine and adenosine salvage	7.85e-03
Purine nucleotide salvage	7.85e-03
5-aminoimidazole ribonucleotide biosynthesis i	1.59e-02
L-lysine biosynthesis ii	1.59e-02
L-homoserine and l-methionine biosynthesis	2.09e-02
L-methionine <i>de novo</i> biosynthesis	2.09e-02
Other amino acid biosynthesis	2.09e-02
Nucleosides and nucleotides biosynthesis	2.25e-02
L-alanine degradation iv	2.97e-02
Udp-α-d-glucuronate biosynthesis (from udp-glucose)	2.97e-02
L-methionine biosynthesis	3.89e-02

Superpathway of purine nucleotides <i>de novo</i> biosynthesis i	4.89e-02
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4 – 35 (downregulated)

Name	P-val
Adenosine ribonucleotides <i>de novo</i> biosynthesis	7.93e-08
Nucleosides and nucleotides biosynthesis	1.02e-07
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	2.46e-06
Purine ribonucleotides <i>de novo</i> biosynthesis	5.30e-06
Ump biosynthesis i	9.58e-06
Purine nucleotides <i>de novo</i> biosynthesis	2.06e-04
Pyrimidine ribonucleotides <i>de novo</i> biosynthesis	2.56e-04
Superpathway of pyrimidine ribonucleotides <i>de novo</i> biosynthesis	2.56e-04
Superpathway of pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis	4.62e-04
Pyrimidine nucleotide biosynthesis	5.63e-04
2'-deoxyribonucleotides biosynthesis	8.66e-04
Pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis	8.66e-04
Pyrimidine nucleotides <i>de novo</i> biosynthesis	8.66e-04
Sugars biosynthesis	1.15e-03
Udp- <i>N</i> -acetyl-d-glucosamine biosynthesis	1.56e-03
Udp- <i>N</i> -acetyl-d-glucosamine biosynthesis i	1.56e-03
Sugar nucleotides biosynthesis	2.40e-03
Udp-sugar biosynthesis	2.76e-03
Carbohydrates biosynthesis	3.13e-03
<i>S</i> -adenosyl-L-methionine cycle	3.68e-03
<i>S</i> -adenosyl-L-methionine cycle i	3.68e-03

L-methionine salvage	3.68e-03
(<i>r,r</i>)-butanediol biosynthesis	5.66e-03
(<i>r,r</i>)-butanediol degradation	5.66e-03
2,3-butanediol degradation	5.66e-03
Fermentation to alcohols	6.43e-03
Homolactic fermentation	6.46e-03
L-methionine biosynthesis	6.46e-03
Fermentation	7.04e-03
Fermentation of pyruvate	9.78e-03
L-methionine biosynthesis iii	1.61e-02
Fermentation to lactate	1.99e-02
Heterolactic fermentation	2.69e-02
Generation of precursor metabolites and energy	2.78e-02
L-cysteine biosynthesis vi (from L-methionine)	3.07e-02
Glycolysis	3.37e-02
Glycolysis iii (from glucose)	3.37e-02
Fermentation to short-chain fatty acids	3.95e-02
Pyruvate fermentation to ethanol	4.04e-02
Gluconeogenesis	4.39e-02
Gluconeogenesis i	4.39e-02
Biosynthesis	4.60e-02
L-methionine biosynthesis i	4.88e-02
Pyruvate fermentation to (<i>r</i>)-acetoin i	4.88e-02
Pyruvate fermentation to (<i>s</i>)-acetoin	4.88e-02

4 – 185 (upregulated)

	P-val
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Name	
Poly(glycerol phosphate) wall teichoic acid biosynthesis	6.60e-06
Teichoic acids biosynthesis	6.60e-06
Cell structures biosynthesis	2.41e-05
Cell wall biosynthesis	2.41e-05
Polyprenyl biosynthesis	1.96e-03
Peptidoglycan maturation	2.71e-03
Purine nucleotide biosynthesis	6.66e-03
Superpathway of pyrimidine nucleobases salvage	9.19e-03
Nucleosides and nucleotides biosynthesis	1.03e-02
Peptidoglycan biosynthesis	1.61e-02
Heptaprenyl diphosphate biosynthesis	1.97e-02
Trna processing	1.97e-02
Adenine and adenosine salvage	2.19e-02
Glycerophosphodiester degradation	2.19e-02
Purine nucleotide salvage	2.19e-02
Utp and ctp <i>de novo</i> biosynthesis	2.19e-02
Pyrimidine nucleotides salvage	2.52e-02
Superpathway of purine nucleotides de novo biosynthesis	3.84e-02
Antibiotic resistance	4.67e-02
Peptidoglycan biosynthesis v (b-lactam resistance)	4.67e-02

4 – 185 (downregulated)

Name	Data
L-histidine biosynthesis	4.26e-05

L-tryptophan biosynthesis	2.11e-04
Adenosine ribonucleotides de novo biosynthesis	5.42e-04
Sugars degradation	6.09e-04
Amino acids biosynthesis	9.01e-04
Proteinogenic amino acids biosynthesis	9.01e-04
Carbohydrates degradation	1.49e-03
L-leucine biosynthesis	2.42e-03
Generation of precursor metabolites and energy	2.80e-03
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis ii	6.80e-03
Fermentation of pyruvate	8.62e-03
Fermentation to alcohols	1.07e-02
5,6-dimethylbenzimidazole biosynthesis i (aerobic)	1.09e-02
Cobalamin biosynthesis	1.09e-02
Fermentation	1.25e-02
Fermentation to lactate	1.33e-02
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	1.48e-02
Pyruvate fermentation to ethanol	2.03e-02
Fermentation to short-chain fatty acids	2.42e-02
Heterolactic fermentation	2.52e-02
<i>n</i> -acetylglucosamine degradation ii	2.73e-02
Glycerol degradation i	2.73e-02
Pyrimidine deoxyribonucleosides degradation	2.73e-02
Chitobiose degradation	2.89e-02
D-galactose degradation i (leloir pathway)	2.89e-02
D-galactose degradation v (leloir pathway)	2.89e-02
Galactose degradation	2.89e-02
Superpathway of pyrimidine deoxyribonucleosides degradation	2.89e-02

<i>s</i>-adenosyl-l-methionine cycle	3.11e-02
<i>s</i>-adenosyl-l-methionine cycle i	3.11e-02
Acetyl-coa biosynthesis	3.11e-02
Fatty acid and lipids degradation	3.11e-02
Glycogen and starch biosynthesis	3.11e-02
Glycogen biosynthesis i (from adp-d-glucose)	3.11e-02
L-methionine salvage	3.11e-02
Polysaccharides biosynthesis	3.11e-02
Pyruvate decarboxylation to acetyl coa	3.11e-02
Sulfur compounds metabolism	3.11e-02
Thiosulfate disproportionation	3.11e-02
Thiosulfate disproportionation iv (rhodanese)	3.11e-02
Glycolysis	4.75e-02
Glycolysis iii (from glucose)	4.75e-02

14 – 35 (upregulated)

Name	Data
Adenine and adenosine salvage	2.42e-03
Purine nucleotide salvage	2.42e-03
L-alanine degradation	1.67e-02
L-alanine degradation iv	1.67e-02
Udp-α-d-glucuronate biosynthesis (from udp-glucose)	1.67e-02
Adenine and adenosine salvage i	3.31e-02
Chitin degradation	3.31e-02
Chitin degradation ii (vibrio)	3.31e-02

Polymeric compounds degradation	3.31e-02
Polysaccharides degradation	3.31e-02
Xanthine and xanthosine salvage	3.31e-02
(<i>s</i>)-malate degradation	4.94e-02
Guanine and guanosine salvage	4.94e-02
Guanine and guanosine salvage	4.94e-02
L-malate degradation ii	4.94e-02

14 – 35 (downregulated)

Name	Data
Adenosine ribonucleotides <i>de novo</i> biosynthesis	9.74e-08
Nucleosides and nucleotides biosynthesis	1.58e-07
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	3.00e-06
Purine ribonucleotides <i>de novo</i> biosynthesis	6.60e-06
Ump biosynthesis	1.11e-05
Superpathway of purine nucleotides <i>de novo</i> biosynthesis i	1.41e-04
Purine nucleotides <i>de novo</i> biosynthesis	2.52e-04
Pyrimidine ribonucleotides <i>de novo</i> biosynthesis	2.95e-04

Superpathway of pyrimidine ribonucleotides de novo biosynthesis	2.95e-04
Superpathway of pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis	5.41e-04
Pyrimidine nucleotide biosynthesis	6.71e-04
Purine nucleotide biosynthesis	8.75e-04
D-galactose degradation i (leloir pathway)	9.45e-04
D-galactose degradation v (leloir pathway)	9.45e-04
2'-deoxyribonucleotides biosynthesis	1.01e-03
Pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis	1.01e-03
Pyrimidine nucleotides <i>de novo</i> biosynthesis	1.01e-03
(<i>r,r</i>)-butanediol biosynthesis	5.94e-03
(<i>r,r</i>)-butanediol degradation	5.94e-03
2,3-butanediol degradation	5.94e-03
L-methionine biosynthesis iii	1.69e-02
Maltose degradation	1.69e-02
Amino acids biosynthesis	2.27e-02

Proteinogenic amino acids biosynthesis	2.27e-02
Sugars degradation	2.77e-02
Carbohydrates degradation	3.56e-02
Nitrogen compounds metabolism	3.59e-02
L-methionine biosynthesis	4.68e-02

14 – 185 (upregulated)

Name	P-val
Pyruvate fermentation to (<i>r</i>)-acetoin ii	5.10e-03
Glycerophosphodiester degradation	1.17e-02
Butanediol biosynthesis	2.15e-02
Poly(glycerol phosphate) wall teichoic acid biosynthesis	2.15e-02
Pyruvate fermentation to acetoin	2.15e-02
Superpathway of (<i>r,r</i>)-butanediol biosynthesis	2.15e-02
Teichoic acids biosynthesis	2.15e-02
Adenosine ribonucleotides <i>de novo</i> biosynthesis	2.67e-02
L-methionine biosynthesis	2.67e-02
Glycerol and glycerophosphodiester degradation	3.47e-02
Glycerol degradation	3.47e-02
L-isoleucine biosynthesis i (from threonine)	3.47e-02
Amines and polyamines biosynthesis	3.51e-02
Ectoine biosynthesis	3.51e-02

L-homoserine biosynthesis	3.51e-02
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14 – 185 (downregulated)

Name	P-val
L-tryptophan biosynthesis	1.15e-06
Chitobiose degradation	9.95e-04
Degradation/utilization/assimilation	1.13e-03
Acetyl-coa biosynthesis	1.92e-03
Pyruvate decarboxylation to acetyl coa	1.92e-03
Sugars degradation	2.38e-03
Carbohydrates degradation	3.92e-03
Ump biosynthesis	4.68e-03
2'-deoxyribonucleotides biosynthesis	1.15e-02
Pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis	1.15e-02
Pyrimidine nucleotides <i>de novo</i> biosynthesis	1.15e-02
Lactose degradation	2.12e-02
Lactose degradation iii	2.12e-02
Fatty acid and lipids degradation	2.44e-02
Pyrimidine nucleotide biosynthesis	3.88e-02
Pyrimidine ribonucleotides <i>de novo</i> biosynthesis	4.07e-02
Superpathway of pyrimidine ribonucleotides de novo biosynthesis	4.07e-02
Aromatic compounds biosynthesis	4.48e-02

28 – 5 (upregulated)

	Data
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Name	
Glycerol degradation i	3.09e-04
Fatty acid and lipids degradation	1.02e-03
Glycerol and glycerophosphodiester degradation	2.12e-03
Glycerol degradation	2.12e-03
Alcohols degradation	5.44e-03
L-alanine biosynthesis ii	1.11e-02
Glycerol-3-phosphate to cytochrome <i>bo</i> oxidase electron transfer	2.22e-02
Maltose degradation	3.31e-02
Aerobic respiration	4.39e-02
Electron transfer	4.39e-02
L-alanine biosynthesis	4.39e-02
Lipoprotein posttranslational modification	4.39e-02
Protein modification	4.39e-02

28 – 5 (downregulated)

Name	Data
Amino acids biosynthesis	1.68e-04
Proteinogenic amino acids biosynthesis	1.68e-04
L-histidine biosynthesis	7.05e-04
L-histidine biosynthesis	7.05e-04
Chitin degradation	3.68e-02
Chitin degradation ii (vibrio)	3.68e-02
Hydroxymethylpyrimidine salvage	3.68e-02
L-cysteine biosynthesis i	3.68e-02

Polymeric compounds degradation	3.68e-02
Polysaccharides degradation	3.68e-02

28 – 35 (upregulated)

Name	Data
Glycerol degradation i	1.35e-03
Acetyl-coa biosynthesis	4.38e-03
Fatty acid and lipids degradation	4.38e-03
Pyruvate decarboxylation to acetyl coa	4.38e-03
Degradation/utilization/assimilation	6.15e-03
Glycerol and glycerophosphodiester degradation	8.98e-03
Glycerol degradation	8.98e-03
Fructose degradation	2.23e-02
Lipoate biosynthesis	2.23e-02
Lipoate biosynthesis and incorporation ii	2.23e-02
Lipoate salvage i	2.23e-02
Melibiose degradation	2.23e-02
Superoxide radicals degradation	2.23e-02
Alcohols degradation	2.24e-02
Glycerol-3-phosphate to cytochrome <i>c</i> oxidase electron transfer	4.41e-02
L-glutamine biosynthesis	4.41e-02

28 – 35 (downregulated)

Name	Data
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L-cysteine biosynthesis i	1.85e-02
Xanthine and xanthosine salvage	1.85e-02
Amines and polyamines biosynthesis	2.76e-02
Ectoine biosynthesis	2.76e-02
Glutathione-peroxide redox reactions	2.76e-02
Guanine and guanosine salvage	2.76e-02
L-homoserine biosynthesis	2.76e-02
Reactive oxygen species degradation	2.76e-02
Adenine and adenosine salvage iii	3.67e-02
Fatty acid biosynthesis initiation i	3.67e-02
Adenine and adenosine salvage	4.57e-02
Purine nucleotide salvage	4.57e-02

28 – 185 (upregulated)

Name	Data
Glycerol degradation i	1.41e-04
Fatty acid and lipids degradation	1.31e-03
5,6-dimethylbenzimidazole biosynthesis i (aerobic)	2.52e-03
Cobalamin biosynthesis	2.52e-03
Glycerol and glycerophosphodiester degradation	4.25e-03
Glycerol degradation	4.25e-03
Alcohols degradation	1.73e-02
Degradation/utilization/assimilation	2.26e-02
D-glucosamine degradation	3.53e-02
Chitobiose degradation	4.96e-02

28 – 185 (downregulated)

Name	Data
S-adenosyl-l-methionine cycle	6.59e-03
L-methionine salvage	6.59e-03
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis i	8.28e-03
Purine ribonucleotides <i>de novo</i> biosynthesis	8.67e-03
Superpathway of adenosine nucleotides <i>de novo</i> biosynthesis ii	1.12e-02
Superpathway of purine nucleotides <i>de novo</i> biosynthesis i	1.23e-02
L-cysteine biosynthesis	1.23e-02
Adenosine ribonucleotides <i>de novo</i> biosynthesis	1.33e-02
Superpathway of branched chain amino acid biosynthesis	1.33e-02
Purine nucleotides <i>de novo</i> biosynthesis	1.78e-02
Amines and polyamines degradation	2.02e-02
Amino acids biosynthesis	3.87e-02
Proteinogenic amino acids biosynthesis	3.87e-02
Purine nucleotide biosynthesis	3.91e-02
Glycine betaine degradation	4.48e-02
Glycine betaine degradation i	4.48e-02
L-cysteine biosynthesis vi (from l-methionine)	4.48e-02
Nad biosynthesis from 2-amino-3-carboxymuconate semialdehyde	4.48e-02